Quality of Norwegian goat milk for cheese production

Kvalitet på norsk geitemelk til osteproduksjon

Philosophiae Doctor (PhD) Thesis

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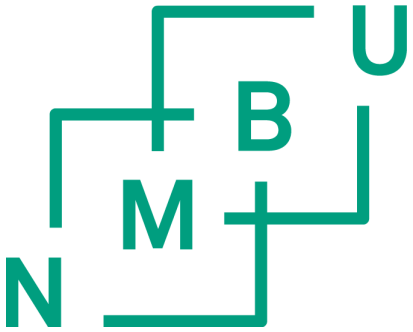

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Contents

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Summary

Inglingstad, R. A. 2016. Quality of Norwegian goat milk for cheese production. Norwegian University of Life Sciences, Philosophiae Doctor Thesis, 2016:19, ISSN: 1894-6402, ISBN: 978-82-575-1348-1

Norwegian goat milk has faced a number of challenges related to the quality of the milk. These challenges have been particularly related to poor rennet coagulation properties and off-flavours. The aim of the project "Quality goat milk for cheese production was to improve the milk quality for cheese production, and this thesis is part of this project.

The purpose of this study was to identify factors influencing the milk properties and composition with emphasis on rennet coagulation parameters and free fatty acids (FFA). The thesis contains five papers from three different experiments.

In the first experiment, milk from goats grazing two different pastures (rangeland and cultivated) was compared with milk from goats that were fed hay indoors. The experiment was conducted twice during the grazing season; early and late, and time of kidding was adjusted so the goats were in the same lactation stage in the two grazing periods. The rennet coagulation parameters were not influenced by pasture type (rangeland or cultivated) or hay, although casein content was higher in milk from goats grazing. However, there was a marked deterioration of rennet coagulation properties in late grazing season and this was observed both in milk from goats on pastures and in those receiving hay. Milk yield was lower in late grazing season (Paper I). There was no difference in the content of FFA in milk from goats grazing rangeland or cultivated pastures or those fed hay, and there was no increase in FFA in late grazing season (Paper II).

In the second experiment, the goats were fed three different types of lipid supplements concentrates: Saturated (palm oil), unsaturated (rapeseed oil) and a control feed without extra lipids. Milk samples were analysed through the entire lactation period, and cheeses were made three times during the lactation. The results showed that the content of FFA and the incidence of rancid and tart flavor of milk were highest in mid lactation but decreased if the goat received the concentrate added rapeseed oil. The composition of fatty acids in milk were affected by type of lipid supplement, and supplementation of rapeseed oil in feed increased the content of unsaturated fatty acids in milk (Paper III). Lipid supplements did not affect the composition of and content of proteins, nor rennet coagulation properties, as these properties were affected by the lactation stage (Paper IV) and genotype (Paper V). Highest casein and

protein content, and the best rennet coagulation- and cheese making properties were obtained in early lactation and when the goats were grazing mountain pastures (Paper IV). Both type of lipid supplement (Paper IV) and genotype (Paper V) affected the cheese quality and -ripening. Supplementation of saturated lipids to the goats gave cheese with better structure than cheese produced from milk of goats that had received unsaturated lipids in the diet.

In the third experiment, we investigated how a genetic defect, which is found in a high frequency in the Norwegian goat herd, influenced rennet coagulation properties and cheese quality. Goats carrying two defective alleles in exon 12 of *CSN1S1* (E12-00) had low levels of α_{s1}-casein and a lower protein content than goats with only one defective allele (E12-01). Furthermore, the cheese produced from milk of goats E12-00 had poorer structure and higher incidence of rancid and tart flavor compared with the cheese produced from milk of goats E12-01 (Paper V).

The main conclusions of this study is that the content of FFA and incidence of rancid and tart flavours in milk is highest when goats are in mid lactation. Furthermore, rangeland pasture did not increase the content of FFA, and the content of FFA was reduced if the goats in mid lactation received concentrate with rapeseed oil. The relationship between the content of FFA and off-flavours was high. TINE's routine analysis of FFA therefore provides a good indication of the milk sensory properties. Supplementation of rapeseed oil in the goats' feed gave a more favorable fatty acid composition of the milk with regard to human nutrition, but rapeseed oil had little or no effect on the milk protein content and rennet coagulation properties. Stage of lactation, and especially genotype, had the greatest influence on composition and content of proteins, FFA, rennet coagulation properties and cheese quality. Further improvement of rennet coagulation properties could be achieved by genetic selection for content of casein and α_{s1}-casein. Development of a concentrate supplemented with rapeseed oil would be beneficial as rapeseed oil reduce levels of FFA, and because it can be produced from Norwegian resources, it is more sustainable compared to palm oil.

The Norwegian goat milk quality has improved due to the last year's efforts in especially breeding and feeding. Norwegian goat milk is of high quality and excellent for the production of cheese.

Sammendrag

Inglingstad, R. A. 2016. Kvalitet på norsk geitemelk til osteproduksjon. Norges Miljø- og Biovitenskapelige Universitet, PhD avhandling 2016:19, ISSN: 1894-6402, ISBN: 978-82-575-1348-1

Norsk geitemelk har hatt en rekke utfordringer knyttet til kvaliteten på melka. Disse utfordringene har vært særlig relatert til svake ysteegneskaper og smaksfeil. Prosjektet «Kvalitetsmjølk for kvit geitost» har hatt som formål å bedre melkekvaliteten med tanke på ysting, og denne avhandlinga er en del av dette prosjektet.

Formålet med denne studien har vært å kartlegge faktorer som påvirker melkas egenskaper og sammensetning med vekt på løpekoagulering og frie fettsyrer (FFS). Avhandlinga inneholder fem artikler fra tre ulike forsøk.

I det første forsøket ble melk fra geiter som hadde gått på to ulike beiter (innmark og utmarksbeite) sammenliknet med melk fra geiter som var fôret innendørs med høy. Forsøket ble utført to ganger i løpet av beiteperioden; tidlig og seint, og kjeingstidspunktet var justert slik at geitene var i samme laktasjonsstadium i de to beiteperiodene. Ysteegenskapene ble ikke påvirka av beitetype (innmark eller utmark) eller av høy, selv om kaseininnholdet var høyere i melk fra geiter som gikk på beite. Derimot var det en markant forverring av ystingsegenskapene seint i beiteperioden, og dette ble observert i melk fra geiter både på beite og hos de som fikk høy. Også geitenes melkeytelse var lavere utover i beiteperioden (Artikkel I). Det var ingen forskjell i innhold av FFS i melk fra geiter på utmarksbeite og innmarksbeite, og det var heller ingen økning utover i beiteperioden (Artikkel II).

I det andre forsøket ble geiter fôret med tre ulike typer fettilskudd i kraftfôret: Metta (palmeolje), umetta (rapsolje) og et kontrollfôr uten tilsatt fett. Melkeprøver ble analysert gjennom en hel laktasjonsperiode, og det ble ystet ost tre ganger i løpet av laktasjonsperioden. Resultatene viste at innholdet av FFS og forekomsten av harsk og besk smak på melka var høyest i midtlaktasjon, men ble redusert dersom geita fikk kraftfôr tilsatt rapsolje. Sammensetninga av fettsyrene i melka ble påvirket av type fettilskudd, og geiter som fikk rapsolje hadde høyere innhold av umetta fettsyrer i melka (Artikkel III). Fettilskudd påvirket ikke sammensetninga og mengde av proteiner, og heller ikke løpekoaguleringsegenskaper, disse egenskapene ble påvirket av geitas laktasjonsstadium (Artikkel IV) og genotype (Artikkel V). Melka hadde best ystingsegenskaper og høyest kasein- og proteininnhold tidlig i laktasjonen og i tida geitene gikk på fjellbeite. (Artikkel IV). Både type fettilskudd (Artikkel

IV) og genotype (Artikkel V) påvirket ostens kvalitet og modning. Tilskudd av metta fett i geitas fôr gav ost med bedre struktur ost ystet av melk fra geiter som hadde fått umetta fett i fôret.

I det tredje forsøket undersøkte vi hvordan en genfeil, som har hatt stor utbredelse i den norske geitebestanden, påvirket ystingsegenskaper og ostekvalitet. Geiter som hadde to defekte alleler i exon 12 i *CSN1S1* (E12-00) hadde lavt innhold av α_{s1}-kasein og lavere proteininnhold enn geiter med kun ett defekt allel (E12-01). Videre hadde ost ystet av melk fra E12-00 geiter dårligere struktur og høyere forekomst av harsk og besk smak sammenlignet med ost ystet av melk fra E12-01 geiter (Artikkel V).

Hovedkonklusjonen i denne studien er at innhold av FFS og forekomst av harsk og besk smak i melka er høyest når geitene er i midtlaktasjon. Utmarksbeite økte ikke innholdet av FFS, og innhold av FFS ble redusert dersom geitene i midtlaktasjon fikk tilskudd av rapsolje. Sammenhengen mellom innhold av FFS og smaksfeil var høy. TINEs rutineanalyse av FFS gir derfor en god indikasjon på melkas sensoriske egenskaper. Tilskudd av rapsolje i foret, gav en mer gunstig fettsyresammensetning i melka med tanke på human ernæring, men rapsolje påvirket i liten eller ingen grad melkas innhold av proteiner eller ystingsegenskaper. Laktasjonstidspunkt, og særlig genotype, har størst påvirkning på melkas sammensetning av proteiner, FFS, ystingsegenskaper og ostekvalitet. Det vil trolig være mulig å oppnå enda bedre ystingsegenskaper dersom det i avlen selekteres for høyere innhold av α_{s1}-kasein og totalt innhold av kasein, da disse faktorene har en positiv påvirkning på ystingsegenskapene. Utvikling av et kraftfôr tilsatt rapsolje til geit vil være positivt både fordi rapsolje gir et lavere innhold av FFS og en mer gunstig fettsyresammensetning i melka, og pga økt bærekraft (i forhold til palmeolje) siden rapsolje kan produseres av norske ressurser.

De siste årene med økt fokus på avl, fôring og helse, har hevet kvaliteten på geitemelka betydelig. Norsk geitemelk er et råstoff av høy kvalitet som er utmerket til produksjon av ost.

Abbreviations

A_{30}	Curd firmness after 30 min
CAE	Caprine arthritis encephalitis
CCP	Colloidal calcium phosphate
CL	Caseous lymfadentis
CSN1S1	Gene encoding α_{s1}-casein
E12-00	Goats homozygous for the Norwegian deletion
E12-01	Goats heterozygous for the Norwegian deletion
ER	Endoplasmatic reticulum
FFA	Free Fatty acids
FFS	Frie fettsyrer
FID	Flame ionization detector
FTIR	Fourier transform infrared
GC	Gas chromatography
GMP	Glycomacropeptide
K_{20}	Firming time
LPL	Lipoprotein lipase
MFG	Milk fat globules
MFGM	Milk fat globule membrane
PCA	Principal component analysis
RCT	Rennet clotting time
SNP	Single nucleotide polymorphism
α-la	α-lactalbumin
β-lg	β-lactoglobulin

List of papers

The thesis is based on the following papers, and are referred to in the text by their roman numerals.

I. Inglingstad, R.A., Steinshamn, H., Dagnachew, B.S., Valenti, B., Criscione, A., Rukke, E.O., Devold, T.G., Skeie, S.B. & Vegarud, G.E. (2014). Grazing season and forage type influence goat milk composition and rennet coagulation properties. *Journal of dairy science*, *97*(6), 3800-3814.

II. Steinshamn, H., Inglingstad, R. A., Ekeberg, D., Mølmann, J., & Jørgensen, M. (2014). Effect of forage type and season on Norwegian dairy goat milk production and quality. *Small Ruminant Research*, *122*(1), 18-30.

III. Inglingstad, R.A., Skeie, S., Vegarud G. E., Devold, T.G., Chilliard, Y. & Eknæs, M. Feeding a supplement rich in unsaturated fatty acids improve lipid composition and flavour in Norwegian goat milk (Manuscript)

IV. Inglingstad, R.A., Eknæs, M., Brunborg, L., Mestawet, T., Devold, T.G., Vegarud, G.E. & Skeie, S.B. (2016) Norwegian goat milk composition and cheese quality: The influence of lipid supplemented concentrate and lactation stage. *International Dairy Journal*, In press, accepted manuscript. Available online 2016.01.08. doi*:* 10.1016/j.idairyj.2015.12.010

V. Skeie, S. B., Inglingstad, R. A., Brunborg, L. J., & Eknæs, M. (2014). The influence of the deletion in exon 12 of the gene encoding α_{s1}-casein (*CSN1S1*) in the milk of the Norwegian dairy goat breed on milk coagulation properties and cheese quality. *Small Ruminant Research*, *122*(1), 50-58.

In addition, the candidate has contributed to the following papers (not regarded as a part of the thesis):

a. Inglingstad, R. A., Devold, T. G., Eriksen, E. K., Holm, H., Jacobsen, M., Liland, K. H., Rukke, E.O., & Vegarud, G. E. (2010). Comparison of the digestion of caseins and whey proteins in equine, bovine, caprine and human milks by human gastrointestinal enzymes. *Dairy science & technology*, *90*(5), 549-563.

b. Eriksen, E. K., Holm, H., Jensen, E., Aabøe, R., Devold, T. G., Jacobsen, M., & Vegarud, G. E. (2010). Different digestion of caprine whey proteins by human and porcine gastrointestinal enzymes. *British journal of nutrition*, *104*(03), 374-381

c. Eknæs, M., Volden, H., Hove, K., Inglingstad, R. A., Bernard, L., Leroux C. & Chilliard, Y. Feeding different lipid supplements throughout lactation in dairy goats: Effects on energy balance and milk production (Manuscript)

1. Introduction

1.1 Milk in human nutrition – a historical perspective

Compared to all other agricultural products, milk is unique in the sense that being *food* is its main and primarily purpose. It contains all the important nutrients needed for the little mammal to develop in the postpartum period. In addition, the content of immunogenic components like immunoglobulins and several other bioactive compounds plays an active protective role. Milk is highly nutritious due to its unique content of fat, proteins, carbohydrates, minerals and minor nutrients. The milk typically consists of mainly water (87 %), lactose (4.6 %), fat (4 %), proteins (3.5 %), minerals (0.7 %) and other elements (like vitamins, organic acids etc.)[(1)]. Milk may at first glance appear to be a homogenous liquid; however, the term "oil-in-water-emulsion" is a more correct description. The fat is dispersed as fat globules in the aqueous phase, and a major part of the proteins are organized into spherical colloidal particles, so-called casein micelles. The unique biochemical structure of milk fat globules and casein micelles are to be described in detail later on.

Surly the ability to ingest milk in adulthood must have been an evolutionary advantage in those days when access to food was limited also in our part of the world. The ability to tolerate milk depends upon presence of the enzyme lactase (or more correctly: lactase phlorizin hydrolase, LPH), which catalyses the hydrolysis of lactose into glucose and galactose. Normally the expression of lactase decreases after the weaning period, however, some part of the human population (approximately 35 %) are able to tolerate milk also after suckling period because the gene encoding lactase is not down-regulated. This phenomena is known as lactase persistence, and the frequency of this trait varies greatly worldwide as shown in Figure 1 [(2)].

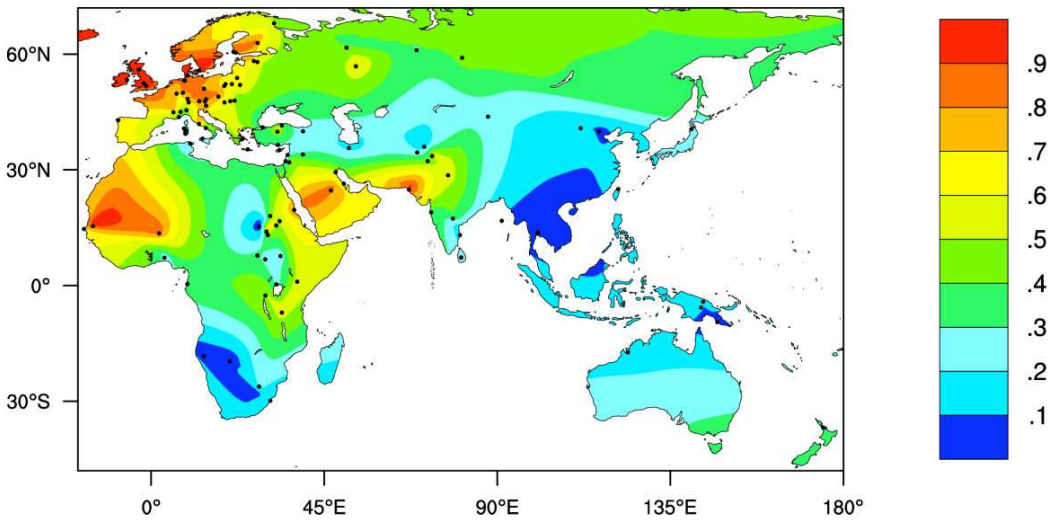

Figure 1. The frequency of lactase persistence in the human population worldwide [(2)]

The differences in distribution of this trait seems to be correlated to dairying [(3)]. By studying an allele that is strongly associated to lactase persistence, it is believed that lactase persistence and dairying coevolved some 7500 years ago in areas somewhere between the central Europe and the central Balkan [(4)]. The finding of whey protein peptides (from β-lactoglobulin) in teeth of several human individuals who lived in Europe and Northern Southwest Asia 5000 years ago [(5)], provides a direct evidence of milk consumption in those areas. However, the milk lipids found in 9000 years old pottery are probably the earliest evidence for dairying [(6)].

1.2 The Goat and its milk

Goats were the first animal domesticated by humans 10 000 years ago, in the highlands near Mesopotamia [(7-9)]. The goat, also referred to as "the poor man's cow", still plays an important role in small-scale households in many developing countries. Compared to cows, goats are superior because the cost of investments and keeping is low, and she produces milk even on marginal feed. The goat is able to utilize plants, herbs and shrubs that are indigestible for humans, in addition she is sure-footed and may live in harsh and rural areas where other animals may not thrive. Goats are often kept by farmers with very little or no land, and are commonly managed by children or women whose survival may depend on their goats [(10; 11)]. The goats are kept for meat or milk production, or as a dual purpose animal. Goat milk contributes to approximately 2 % of the total milk production in the world [(12)]. The total number of goats counts 976 millions [(13)], of which the population in Asia, Africa and Latin-

America contributes more than 95 % [12; 13] (Figure 2). While the number of goats in Europe only contributes to less than 2 % of the world's goat population (Figure 2), Europe produces about 20 % of the total goat milk [10] and 45 % of the goat milk cheese [13]. Most of the goat milk and –cheese production in Europe is located in the Mediterranean countries In these countries goat milk products are highly valued, and milk production is often supported by governmental grants [7].

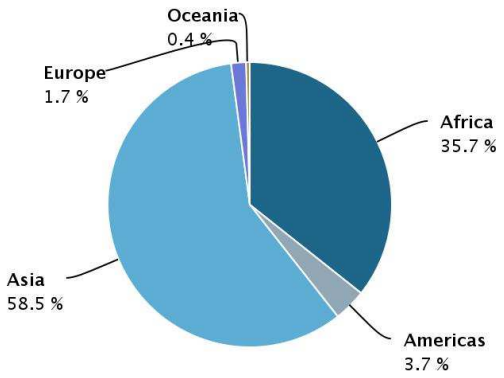

Figure 2. Distribution of goats in the world (2013)[13].

1.3 Norwegian goats and milk production

Most goats in Norway are dairy goats, however, the number of goats for meat production is slightly increasing [14]. The number of Norwegian goat farms counts 300 and the average herd size is just above 100 animals. The goat milk production is largest in the northern and western part of the country [14]. The production is seasonal with kidding in January-March, and almost no milk is produced from November to January [15]. Due to the harsh climate in Norway, pastoral production is only possible during the summer months, while the goats are fed indoor most of the year. Some farmers still utilizes mountain pastures, and the goat's browsing is important to avoid areas to become overgrown [16]. The goats graze to a large extent natural unimproved grassland or free range in forest and mountain grasslands. The quality of these pastures is variable and declines during the grazing season [17]. Low prices of concentrates has lead to a more intensive production less dependent of pastures. This production system increases in Europe [10], however in Norway farmers receive grants for pastoral production [14].

Keeping domesticated goats has long traditions in Norway. In the middle of the 19.th century, the total number of goats counted 350 000 heads [15], today the herd counts only 33 000 animals [14]. While the number of goats and producers are declining, the milk production has been stable the last 20 years (Figure 3). Compared to other Scandinavian countries, the number of dairy goats in Norway is relatively high [13].

Most of the produced milk is sold to the Norwegian dairy cooperative (TINE), while only a small proportion is processed at the farm. About 70-80 % of the milk is used for production of Brunost (a sweet whey "cheese") and 6 % of the milk is used for production of the spreadable cheese Snøfrisk [15]. The sales and consumption of Brunost is declining, while the demand for chevre and feta –type of cheeses is increasing. The consequence is a surplus of goat milk, and development and marketing of new goat milk products is required. However, until recently, the milk has been of variable quality, both regarding off-flavours and poor coagulation ability [18]. This has caused a delay in development of new products, and has created (and maintained) a reputation among consumers of tart and rancid goat milk products.

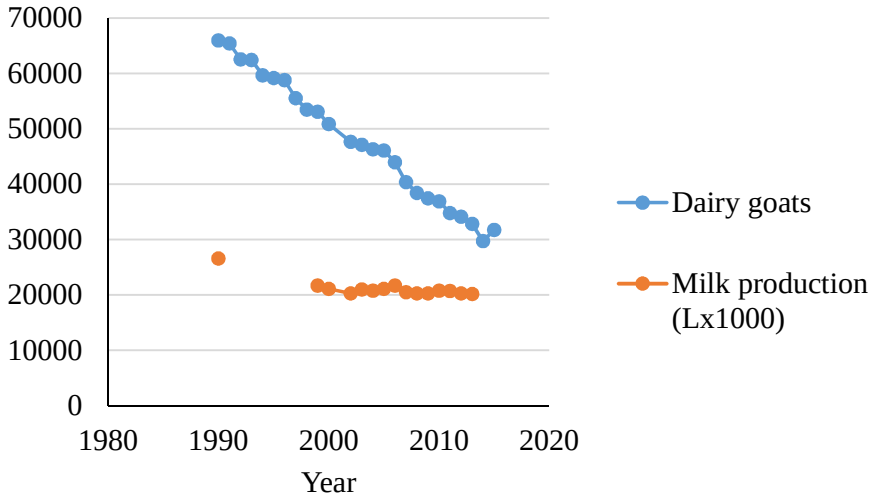

Figure 3. Goats and goat milk production in Norway 1990-2015 [19]

1.4 Milk composition and technological parameters

1.4.1 Proteins

The protein content in milk varies with factors like species [20], breed [21], genotype [22], lactation stage [23] and feed [24]. The protein content in goat milk is similar to that of cow milk and is reported to vary from 2.6-4.8 % [21; 25]. The major whey proteins are α-lactalbumin (α-la) and β-lactoglobulin (β-lg), while the casein fraction consists of α_{s1}-, α_{s2}-, β- and κ-casein and together these proteins comprise more than 95 % of the total protein content of milk. Several minor proteins present in milk include immunoglobulins, serum albumin, lysozyme, lactoferrin, transferrin, prolactin, lactoperoxidase, lipoprotein lipase (LPL) among others [26; 27]. The ratio of α_{s1}-, α_{s2}-, β- and κ-casein in cow milk is roughly 4:1:4:1, while the proportion of β-casein is higher in goat milk compared to the cow [27-29]. α_{s1}-casein was earlier reported to be totally absent in goat milk [27], but later it was shown that the content of α_{s1}-casein was extremely variable due to the polymorphism at the α_{s1}-casein locus [22; 30; 31].

1.4.1.1 Caseins and casein micelles

The casein monomers; αs1-, αs2-, β- and κ-casein, have unique molecular properties that explain why they are present in an aggregated form, the casein micelles, in the milk. The caseins have an uneven distribution of charged and hydrophobic amino acids in the primary sequence, which gives rise to their amphiphilic nature. Some secondary structures exists [32], but is rather low due to a high content of prolines. Moreover, their structure is open and flexible as the amino acid cysteine is totally absent or present as only in minor quantities, hence the possibility to stabilize their tertiary structures by disulphide bridges is limited. α_{s1}-, α_{s2}- and β-casein are referred to as calcium sensitive as they precipitate in concentrations of calcium above a certain level. Calcium interacts with their phosphorylated serine residues presented in clusters along the primary sequence. κ-casein is different in this aspect, and this is why this casein is located at the exterior of the casein micelle [33].

Their main function of the casein micelles, in addition to supply essential amino acids and nitrogen, is transport of calcium phosphate from the mammary gland to the little infant. At the natural milk pH (6.7) the calcium phosphate has low solubility, and if it was not for these unique transport vehicles, the casein micelles, calcium phosphate would precipitate in the mammary gland [34; 35]. The structure of the casein micelle in cow milk has been investigated over the last 70 years, and different models have been proposed [33; 36-46].

Nanoclusters of colloidal calcium phosphate (CCP) serves as interlocking points in the interior of the casein micelle [40; 44]. In addition, the caseins are linked together via hydrophobic interactions [47]. β- and κ-casein each have one hydrophobic region, and α_{s1}-caseins have two closely located hydrophobic regions, while α_{s2}-caseins have two (or three) hydrophobic regions [47]. The β- and α_{s1}-caseins acts as chain extenders, while α_{s2}-casein acts as a branch point as it contains two main hydrophobic regions and one or more phosphoserine cluster(s) in the casein micelle formation [44; 47]. Because κ-casein does not contain phosphoserine clusters, and its C-terminal is hydrophilic, it can only interact with the other caseins through its hydrophobic region. In this way, the κ-casein acts as a chain terminator, with its hydrophilic, glycosylated and negatively charged C-terminal protruding into the solvent [47]. This features of the κ-casein gives the impression of a "hairy" casein micelle [33], and its concentration is negatively proportional to the size of the casein micelles [48]

Most models agrees upon an network of β-and α-caseins, in addition to nanoclusters of calcium phosphate in the interior of the casein micelle, and κ-casein located on the surface with its hydrophilic, glycosylated and negatively charged C-terminal protruding into the solvent [33; 44; 45; 49; 50]

The most recent model of Dalgleish [45; 49] is presented in Figure 4.

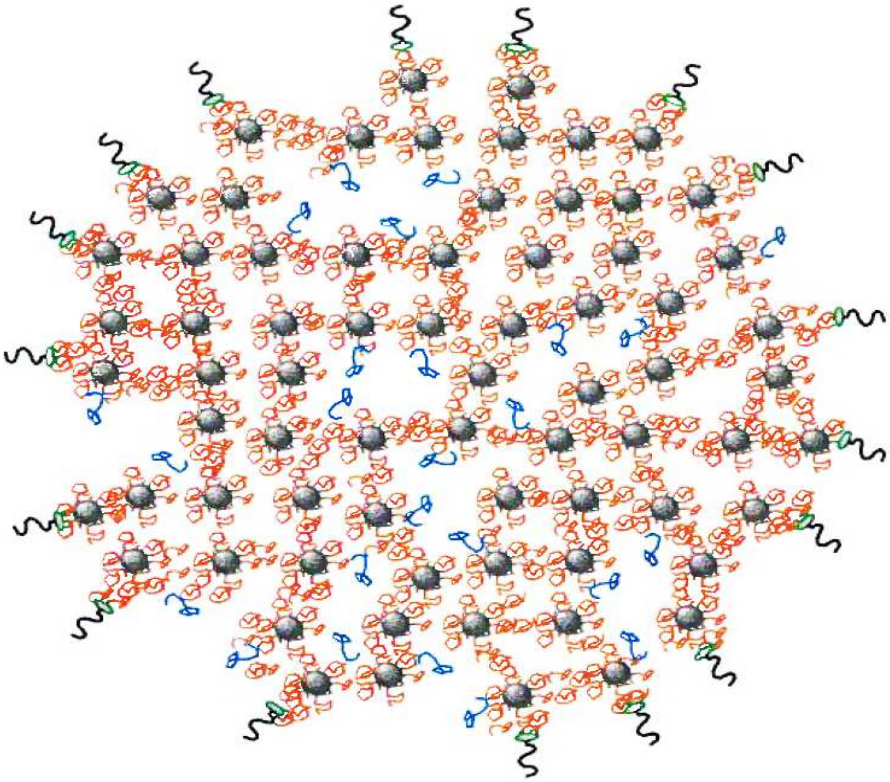

Figure 4. A recent model of the casein micelle. The interior consists of nanoclusters of calcium phosphate (grey) and α- and β-caseins (red). The "hairy layer" on the surface consists of κ-casein. Some of the β-caseins (in blue) is susceptible for leaking out of the casein micelle upon cooling because of more loose interactions in the casein micelle. The components are not to scale [(49)]

1.4.2 Rennet coagulation of milk

The main and original purpose of casein micelles is transportation and delivery of essential amino acids, nitrogen and minerals, especially calcium and phosphate, to the infant. When the casein micelles enter the acidic environment in the stomach, calcium and phosphate is released due to the low pH. Moreover, chymosin, the main gastric enzyme of newborn ruminants, specifically cuts off the glycomacropeptide (GMP) at position Phe105-Met106 of the κ-casein. Both the low pH and the loss of GMP destabilises the casein micelles and they form a clot in the stomach. In this way, a prolonged delivery of proteins to the gut becomes possible. It is this principle of destabilisation of the casein micelles we utilize in the very first step of the cheese making process.

Rennet is an extract of digestive enzymes from the fourth stomach (the abomasum) of unweaned calves. Rennet made from stomachs of young calves contains a higher proportion of chymosin, and the proportion of pepsin increases with the age of the animal [51]. Rennet or acid (usually produced by lactic acid bacteria), or a combination of both is used to destabilize the casein micelles in the cheese making process.

At the natural pH of milk, the casein micelles cannot approach each other due the negative net charge and steric stabilization provided by the κ-casein (Figure 5 A). The chymosin hydrolyses κ-casein between the phenylalanine-105 and methionine-106 residues and releases the negatively charged part of the κ-casein, the glycomacropeptide (GMP) into the whey. The loss of the GMP causes a gradual decrease in zeta potential and electrostatic repulsion between the casein micelles (Figure 5 A) [47; 49]. The removal of the steric hindrance (GMP) of the casein micelles allows new interactions (mainly hydrophobic) between the micelles which leads to aggregation. The aggregation do not start until the removal of GMP is almost complete, and small snowflake-like aggregates becomes visible in the milk and the viscosity increases [49; 52]. This is referred to as the rennet clotting time (RCT) [52], and is further described in 1.4.2.1.

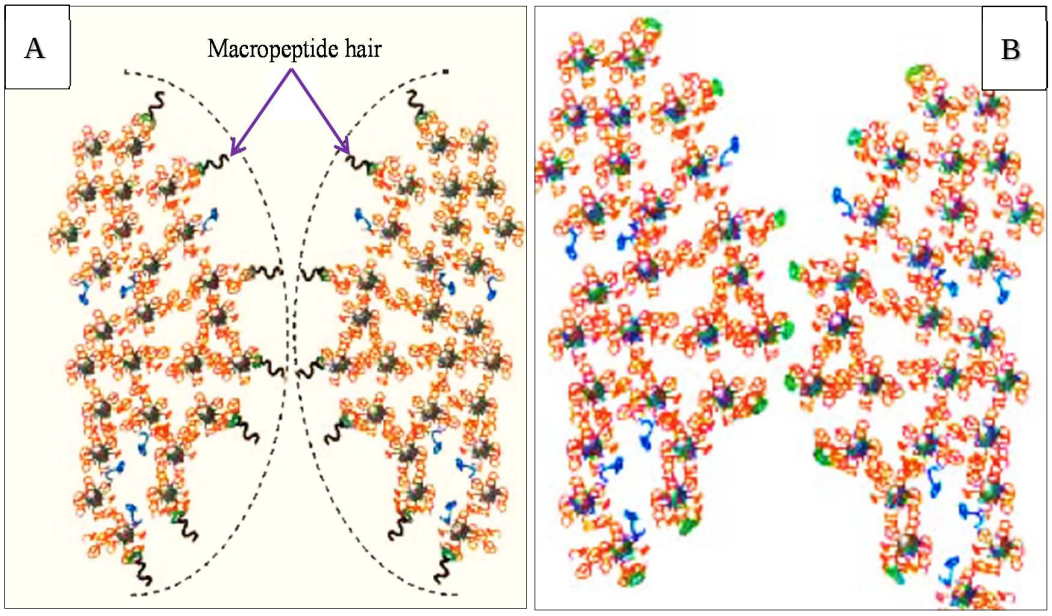

Figure 5. Native casein micelle with intact κ-casein providing charge and steric stabilisation against aggregation (A), and casein micelles that have lost the glycomacropeptide of the κ-casein due to rennet induced hydrolysis(B) [49]).

No aggregation occurs below 15°C or without presence of calcium, whereas a decrease in pH leads to shorter RCT and increased gel firmness [52]. At the initial stages of aggregation, hydrophobic interactions between the rennet-destabilized micelles is the main force. As the gelation proceeds, the colloidal calcium phosphate (CCP) becomes increasingly important in the creation of the gel network [52]. A continuous network entraps whey, fat globules and microbes within. Rennet induced gels tends to stick to the cheese vat and do not contracts notably if not wetted or disrupted by cutting. However, upon cutting, the gel network shrinks and expels whey. This process is called *syneresis* [1]. During the syneresis, the number of interactions in the gel network increases, and the pore size of the gel gets smaller and the moisture content decreases. Rennet induced gels are capable of obtaining a much lower moisture content compared to acid induced gels, and this is most probably due CCP interactions, as acids precipitated casein micelles do not contain CCP [52].

1.4.2.1 Measuring rennet coagulation properties.

The formagraph method is a relative rapid method for measuring rennet coagulation properties. The method is based on the movement of pendulums immersed in linearly oscillating samples of milk. As long as there is no increase in viscosity of the milk, no force is applied to the pendulums, and hence they do not move. When the viscosity increases, the pendulums move because of the drag force applied to them. As coagulation proceeds the applied force on the pendulum increases in which increases its amplitude. The movements are registered, and the firmness versus time is recorded as a diagram (Figure 6). The split of the diagram is the time point where the pendulums starts to move; *rennet clotting time* (*r* or ***RCT***) of the milk. The time in minutes from RCT to a gel firmness equivalent to 20 mm amplitude is called the *firming time*, k_{20}. The amplitude obtained in mm of the diagram after 30 min is equivalent to the *curd firmness* after 30 min, a_{30} (Figure 6) [53].

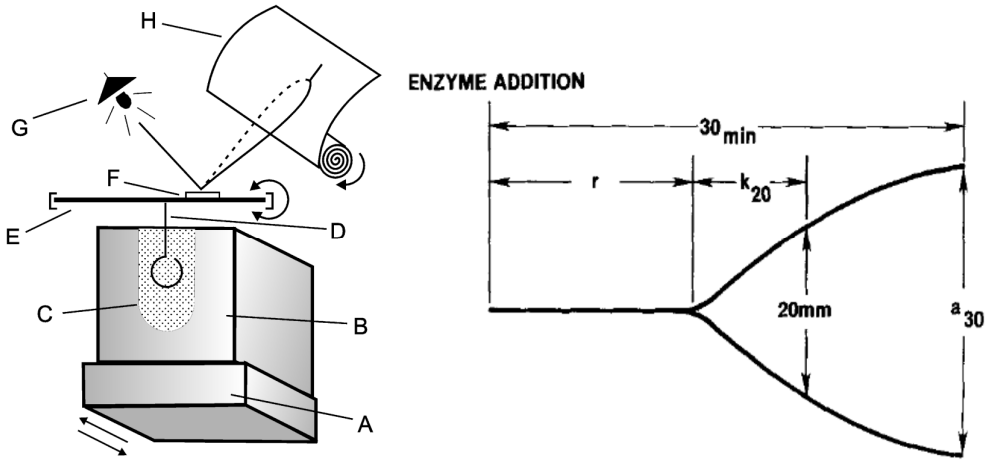

Figure 6. Illustration of the recorder unit of the Formagraph to the left. A: oscillating plate, B: sample block, C: milk sample, D: pendulum, E: girder attached to the pendulum, F: mirror, G: light, H: recording paper. A typical diagram with the milk coagulation parameters obtained from the formagraph is shown to the right. Modified figures from [53; 54].

1.4.3 Lipids

The main purpose of milk lipids is to serve as energy source of the neonate, and the fat content varies greatly among species depending of the need of the respectively progeny. The fat content of goat milk is somewhat similar to that of cow milk; ~4 %, but like cow milk, it varies with factors like stage of lactation, milk yield, feeding, breed etc. [55]. Milk lipids are a source of essential fatty acids (like omega-3 fatty acids), and the milk fat is important for the rheological properties of dairy products. Goat milk lacks agglutinin, and therefore, in addition to smaller milk fat globules, displays a slower creaming rate compared to cow milk. Moreover, goat milk do not contain β-carotene, and the fat of goat milk therefore appear much whiter than the fat of cow milk [55; 56]. More than 400 different fatty acids are identified in cow milk [57], and goat milk most likely has a comparable number of fatty acids. Several of these fatty acids may serve as precursors of both favourable and unfavourable flavour compounds [55]. Most of these 400 fatty acids are found in very low concentrations, including different varieties of branched chain fatty acids (BCFA). Several of those are known to have a very low oral detection thresholds [58], and these are reported higher in goat milk compared to cow milk [59].

1.4.3.1 Lipid synthesis and structure of the milk fat globules

Fatty acids in ruminant milk originate partly from *de novo* synthesis in mammary gland and partly from lipids from feed or adipose tissue. Short and medium chain fatty acids with less than 16 carbons and some of the C16s are synthesized *de novo* by using acetate and β-hydroxybutyrate as substrate. Longer fatty acids originate from the diet and adipose tissue [55]. Compared to the fatty acids composition of cow milk, the goat milk contains a higher proportion of the fatty acids C6, C8 and C10 [60; 61] and *Capra* (goat) is reflected in the trivial names of these fatty acids: Caproic-, caprylic- and capric acids, respectively. The milk lipids are secreted as milk fat globules (MFG) into the milk. The diameter of cow MFG size vary from approximately 0.1-15 µm (reported range of goat MFG is 0.73-8.58 µm [62]). The content of small fat globules in milk is high, however they comprise only a small part of the total fat content [1]. A trilayer membrane covers the triglyceride core of the MFG (Figure 7A). When the triglycerides are released from endoplasmatic reticulum (ER), the microlipid droplets are covered by the ER monolayer membrane. In cytosol, the microlipids droplets fuse with each other, and therefore their volume increases on their way to the apical cell membrane. The lipid droplets are enveloped by the plasma membrane of the mammary gland epithelial cells when they are secreted as milk fat globules into the lumen (Figure 7 B) [55; 63; 64].

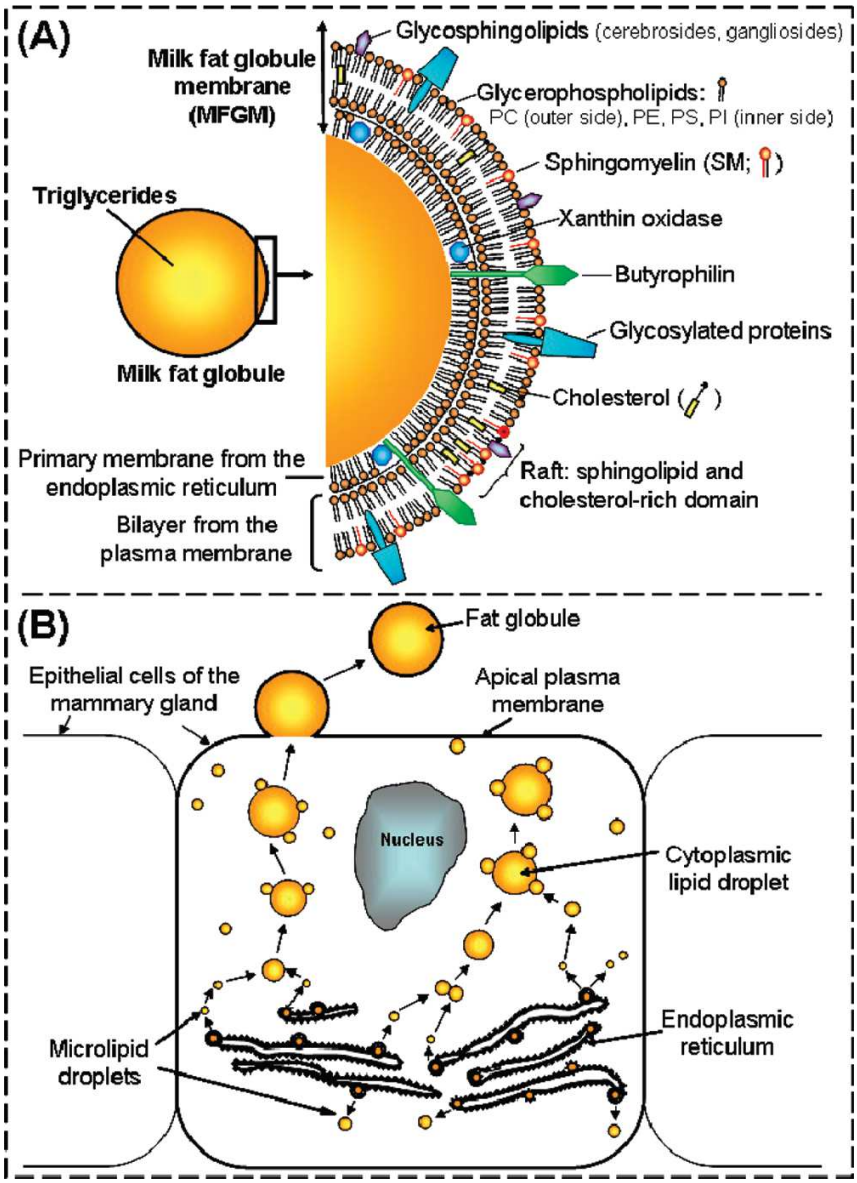

Figure 7. The structure of the milk fat globule membrane (A). Synthesis and secretion of milk fat globules (B) [65].

1.4.3.2 Lipolysis

The enzymatic hydrolysis of milk lipids is called *lipolysis.* The subsequent release of free fatty acids (FFA) is responsible for hydrolytic rancidity or lipolysed flavour in milk [66]. Addition of FFA with less than 14 carbons were found to give the strongest contribution to rancid flavour in cow milk [67]. The enzyme responsible for the lipolytic activity in milk is called *lipoprotein lipase* (LPL). LPL hydrolyses fatty acids from the position *sn*-1 and *sn*-3 from the bovine triglycerides. Bovine LPL is a very potent lipase with pH optimum around 9

and a temperature optimum of 37 ℃ and is capable of turning the milk rancid within 10 min [68; 69]. However, due to its association with the casein micelles (in bovine milk) and the protective MFGM enveloping the triglycerides, very little lipolysis normally occurs. Cow milk that is prone to high degree of lipolysis appears to have a higher level of activation factors like apolipoproteins or lower levels of LPL inhibitors like proteose peptone fraction PP3 than normal milk [68; 69]. Similar factors of LPL activation and inhibition is also reported in goat milk [61], but those are not extensively investigated.

Contrary to cow milk, only 8 % of the lipolytic activity is found in the casein fraction, while the activity was primarly detected in the cream (46 %) and the serum (46 %) phase in goat milk [70]. The association of LPL with the fat globules in goat milk rather than the casein micelles may explain the higher correlation between LPL activity and FFA in goat milk compared to cow milk [61].

As the potential of LPL is much higher than the actual lipolysis, the LPL's accessibility to its substrate (the triglycerides) is most likely an important factor. Therefore, a different composition of the MFGM of susceptible samples may explain the different degrees of lipolysis between samples [71; 72]. A recent comparative study of the proteins of MFGM in different species revealed that goat MFGM has a remarkably higher content of xanthine dehydrogenase/oxidase, stomatin and MAP34-B protein compared to MFGM of cow, human and yak milk [73]. Interestingly, a higher content of stomatin in addition to lactadherin, was reported in goats with low or no synthesis of α_{s1}-casein (homozygous for null alleles, O/O) compared to goats with high synthesis of α_{s1}-casein (homozygous for the strong alleles, A/A) [74]. Whether the differences in the protein composition of the MFGM affects lipolysis in goat milk remains unknown, and warrants further investigations.

1.5 Challenges and strategies to improve Norwegian goat milk quality

1.5.1 Milk protein polymorphism and milk quality

A single nucleotide polymorphism (SNP) in exon 12 of the gene encoding α_{s1}-casein (*CSN1S1*), is detected in the Norwegian goat population. Three different alleles of this SNP is present in the Norwegian dairy goat heard, and one of the alleles carry a deletion of one of six consecutive adenosines. This allele was first described by Lien [75] after a collaboration with the French pioneers in this field (Grosclaude, Mahé and Martin) in the early nineties [15]. The deletion leads to a premature stop codon [75-77], and the truncated protein not detectable

by isoelectric focusing (IEF) [78]. The frequency of this defective allele was extremely high (73 %) in the Norwegian goat population [77; 79]. Goats that carry two alleles of this genotype are denoted E12-00, heterozygous goats as E12-01 and non-carrier goats as E12-11. E12-00 goats have a higher content of FFA [80], lower casein content and longer RCT and k_{20} and weaker a_{30} [80; 81] compared to E12-01 or E12-11 goats.

Even though it was known during the early nineties that the number of E12-00 goats ("null"-goats) was high [75; 78], it took several years before it was implemented in the breeding programme and performance studies of Norwegian goats. An effort to reduce the frequency of the defective allele started in 2008, when all farmers were offered to have their bucks genotyped. When the first genotyping of bucks started in 2005, the frequency of the defective allele among the bucks was as high as 80 %, while in 2012 it was reduced to 16 % [82]. The (unofficial) allele frequency among genotyped licenced bucks (382) is now (in 2015) 5 % [83]. A high frequency of a null allele of α_{s1}-casein is recently reported in goats of the Swedish Landrace, and may be the same type of polymorphism as in Norwegian dairy goats as Norwegian bucks have been used for breeding in Sweden [84; 85].

It has been suggested that α_{s1}-casein plays an important role in transport of caseins from the endoplasmatic reticulum (ER)[86]. Accumulation of immature caseins in ER cisterna is observed in goats with reduced synthesis of α_{s1}-casein [86], and this has been suggested to also influence secretion of other milk components [87]. Moreover, polymorphism at *CSN1S1* locus has been shown to affect gene expression of several genes influencing lipid synthesis and secretion, membrane fluidity and cell interactions [88]. Recently, polymorphism at *CSN1S1* locus was shown to affect the membrane composition of the milk fat globules (MFG) [74]. This may explain why goats with no or low levels of α_{s1}-casein including the E-12-00 goats are different concerning other milk components.

1.5.2 Goat flavour and off-flavours

In the early sixties, there was raised a concern about the decrease in distinctness of flavour in the Norwegian goat milk [89]. Rønningen (1965) studied factors related to variation in goat milk flavour, and found that flavour intensity was related to higher milk yield but lower milk fat content. Therefore, he suggested selection for a high milk yield in order to increase the flavour intensity of the goat milk [89]. Selection experiments for increasing goat milk flavour started in 1969 at the former Agricultural University of Norway [90; 91], and after five generations the flavour intensity, content of palmitic acid and FFA and milk yield had

increased, and the fat content decreased [92]. Many years later, a positive correlation of milk yield and FFA and (too) strong flavour, and a negative correlation to fat, protein and lactose, similar to what was reported by Rønningen [89] and Skjevdal [92], was found. However, because of new technology and knowledge, Dagnachew et al. were able to link these traits to a specific SNP [79]. This SNP (called SNP 14 in [79]) is the position of the aforementioned deletion in exon 12 of the gene encoding α_{s1}-casein, and therefore the selection for strong flavour and high milk yield may explain the high frequency of this genotype among the Norwegian dairy goats. However, during the years between the selection for a stronger flavour and the confirmation of the genetic link between strong flavour and low content of α_{s1}-casein, the flavour had become too strong or the flavour preferences among consumers had changed [93]. While 'strong flavour' was regarded as positive in 1965, 'strong flavour' caused problems in later years [18]. One can wonder if the strong flavour was regarded as only positive when reading the first line of the review by Skjevdal (1979): "The specific flavour of goat's milk is undesirable for direct consumption but for cheese production its presence can be advantageous" [92]. During the eighties and nineties, the flavour was so prominent that some of the goat cheeses were withdrawn from the market (Knut Erik Grindaker, pers.comn 2014). Whether this was due to increase in flavour, change in the consumers preferences or a combination of these factors is uncertain. However, in order to improve the flavour, "goat flavour" was defined as something different from tart and rancid flavour [93]. The tart and rancid flavour are often related to content of free fatty acids, while the origin to the goat flavour is more complex and not fully known. The goat flavour should be characteristic "goaty", but without tart or rancid off-flavours. The tart or rancid flavour may have been misinterpreted as goat flavour because the goat milk are more likely to develop such flavours [93]. Moreover, the lipolysed flavour caused by FFA is also sometimes described as "goaty", "soapy", "bitter" or "butyric". However, these descriptions are regarded as ambiguous because they may be caused by other reactions than lipolysis [66].

Sensory evaluation of goat milk as basis for payment started in 2002. Measurement of FFA was included in the routine control in 2003 in addition to sensory evaluation. From 2008, measurement of FFA replaced sensory evaluation as basis for payment to the farmers. From 2014, the producers got a deduction in payment if content FFA exceeded 1.6 mM [94]. The focus of reducing FFA levels has led to a decrease of average annual FFA content form 1.53 in 2005 to 0.63 mM in 2012 (Helga Kvamsås and Kunt Erik Grindaker, pers.comn).

1.5.3 Healthier Goats- Eradication of common goat diseases

Up to recent years, the goat population has faced challenges due to a high frequency of contagious bacterial and viral infections. The three essential diseases are 1) Caprine arthritis and encephalitis (CAE), 2) Caseous lymfadentis (CL) ("byllesjuke") and 3) Para tuberculosis/Johne's disease. Antibodies against the CAE virus was detected in nearly 90 % of the goat population (in 1998) [95]. The diseases are regarded at chronic, with no efficient treatment or vaccine available. In 2001, the project "Healthier Goats" were initiated with the goal of eradicate CAE [96]. A method called "snatching" (snapping) was developed to establish a new healthy goat herd. Once born, the kid is immediately taken away from its dam and raised separately from the herd. The old goats are all slaughtered and the production continues with the snatched kids after extensive cleaning of the barn, surroundings and equipment [97; 98]. This procedure is laborious, and may resist farmers from eradication of their herds [99], however the healthier goats produces more milk [98; 100]. The "Healthier goats" project proved to be successful, and from 2013, all goat milk delivered to TINE came from CAE free herds [96]. At present, CAE, CL and caprine paratuberculosis are eradicated from the Norwegian dairy goat population. This has lead to an increase in both milk yield [100] and the goat's welfare [101].

2. Background for the project "Quality goat milk for cheese production" and aims of current study

As described in the previous chapter, there were several challenges regarding the Norwegian goat milk at the turn of the century. The milk had quality problems regarding impaired rennetability and off-flavours, and a large proportion of the goats were infected with various diseases (see section 1.5). What about the large proportion of the "null"-goats? Were they the unique and true Norwegian goats and their flavour a reflection of the rough Norwegian nature? Maybe their poor rennet clotting ability was an indication of a more digestible milk, and maybe people allergic to cow milk could tolerated it [102]? The digestibility of the milk was examined, and even goat milk proved to have some positive bioactive properties [103; 104], there was no difference between the "null"-goats and those with a higher content of α_{s1}-casein [105]. Nevertheless, it was decided to improve the goat milk with regards to cheese production rather than marketing the milk as a "health-food". The genetic testing of bucks for breeding started in 2008, and the project "Healthier goats" with the goal of eradication CAE was running. The same year, a large project involving recourses in breeding, feeding, forage, physiology, milk and cheese quality got founded. This project was called "Quality goat milk for cheese production" and the aim was to "Establish breeding and feeding strategies that ensure optimal and stable goat milk quality for consumer preferred cheese products" [106]. The project was a collaboration between NMBU (formerly UMB), Bioforsk, Tine BA and the Association of Sheep and Goat Breeders (NSG), and I got a possibility to study goat milk quality for my PhD thesis within this project.

The aims of this thesis were to increase the knowledge of:

- Effect of pasture on milk quality and composition in early and late grazing season.
- Effect of palm oil vs rapeseed oil on milk quality and composition and cheesemaking parameters
- The influence of genotype at *CSN1S1* locus on rennet coagulation and cheesemaking properties of goat milk
- Factors influencing the levels of FFA
- Factors influencing rennet coagulation properties

3. Experimental design

The goat milk used in these studies were collected from two different goat heards (Gibostad (A) and Ås (B and C) at three different locations in Norway: From the university farm (Ås), from Gibostad research centre (Troms) and the mountain pasture at Folldal (Ås-goats) (Figure 8).

The study is based on three main experiments and the results are presented in five papers:

A) *Effects of forage and grazing season on milk production, composition and quality and rennet coagulation properties*

Paper I

Paper II

B) *Effects of lipid supplemented concentrate on milk quality, rennet coagulation and cheesemaking properties*

Paper III

Paper IV

C) *Effects of CSN1S1 genotype on rennet coagulation and cheese making properties*

Paper V

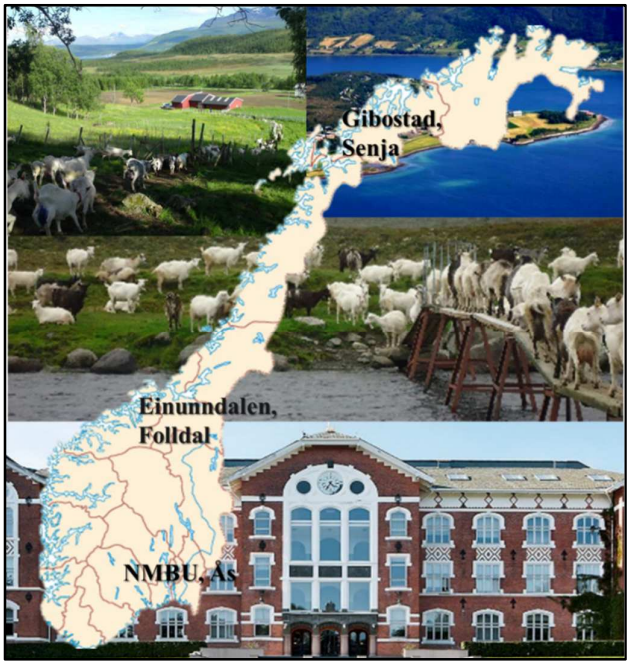

Figure 8. Goat milk was collected from Gibostad in the north (Paper I & II), from the mountain pasture in Einunndalen (Paper III and IV) and at the University farm (Paper III, IV and V). Photos by K. Hansen, www.botnhamn.no, R. Inglingstad, www.statsbygg.no.

A) Effects of forage and grazing season on milk production, milk quality and rennet coagulation properties

The objective was to study the effects of grazing season (early (EGS) and late (LGS)), forage type (hay (high (HH) and low (HL)quality hay) and pasture (cultivated (PC) and rangeland (PR))) on milk composition and rennet coagulation properties. To separate the effects of these factors from the effect of lactation, all goats were in the same lactation stage at the start of the feeding experiment. This experiment took place at Gibostad research centre, at the island Senja in Troms (Figure 8). Eighty goats were divided in two groups; EGS and LGS, and the goats in EGS kidded 8 weeks before the goats in LGS (Figure 9). At ~130 days in milk, (28th of June and 16th of August for the EGS and LGS group, respectively), the goats were randomly assigned to four forage treatment groups: PC = cultivated pasture, PR = rangeland pasture, HH = High quality hay and HL= Low quality hay (Figure 9). Milk was collected one week before and two weeks after onset of forage treatment (Indicated on Figure 9).

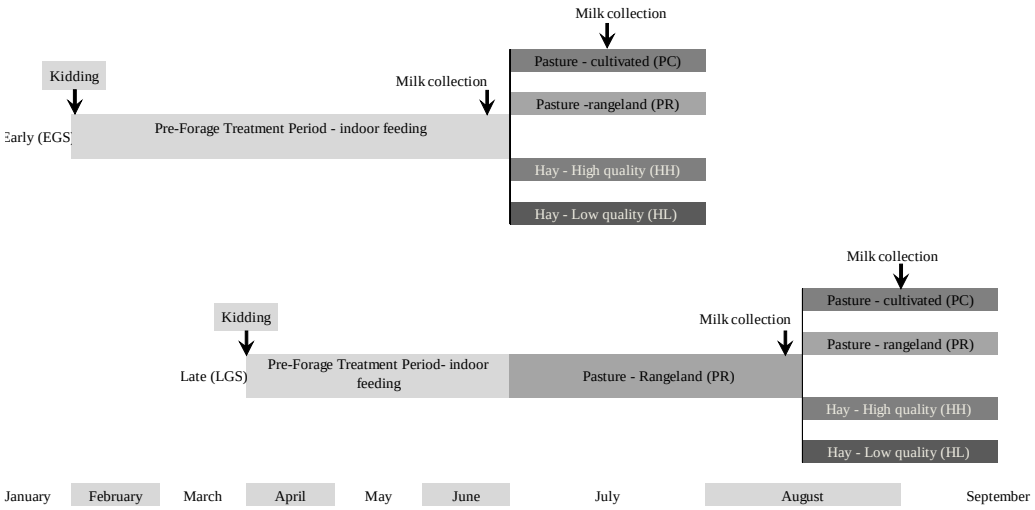

Figure 9. Experimental design used in Paper I and II. Figure taken from [107].

B) Effects of lipid supplemented concentrate on milk composition, rennet coagulation and cheesemaking properties.

The objective of this feeding experiment was to study the effect of feeding a concentrate supplemented with either rapeseed oil (UNSAT) or palm oil (SAT) compared to a control concentrate with no extra fat (CONTROL). Thirty goats were fed the control concentrate until 60 DIM, thereafter they were divided in three groups, and fed one of the three different

concentrates throughout the lactation cycle according to Figure 10. Milk was collected at 30, 60, 90, 120, 190 and 230 days in milk (DIM). Cheese was produced from bulk milk from the ten goats in each group at 90, 120 and 190 DIM. Cheese samples were taken from unripened cheese and cheese ripened for 2 and 4 months. The goats were located at the University farm at Ås, apart from June-September (~130-200 DIM) when the goats were grazing mountain pastures in Einunndalen in Folldal (Figure 8).

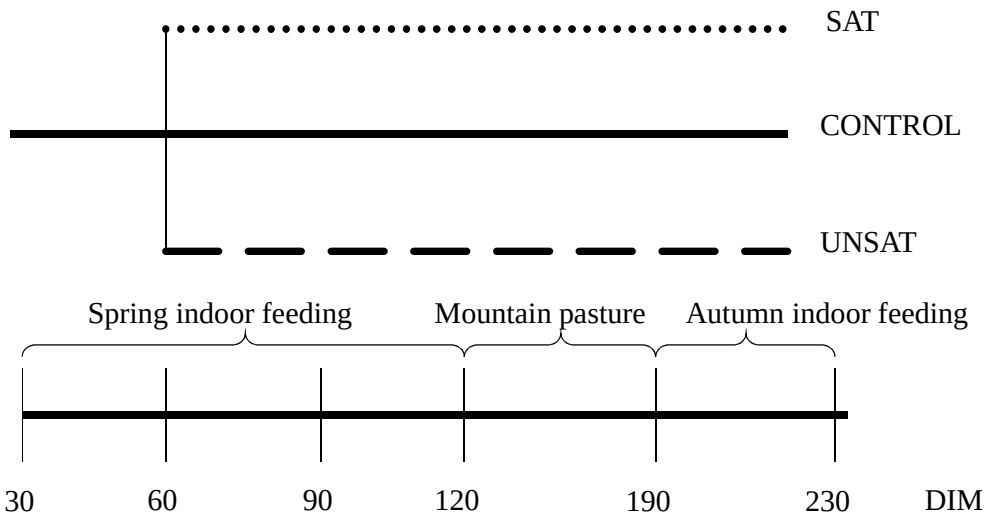

Figure 10. Experimental design for study B, Paper III and Paper IV

C) Effects of CSN1S1 genotype on rennet coagulation and cheese making properties

The aim was to investigate differences in casein composition and cheesemaking properties between goats homozygous (E12-00) or heterozygous (E12-01) for the deletion in exon 12 at the *CSN1S1* locus. Milk was sampled at 30 and 60 DIM, and cheese samples were analysed in unripened cheese and cheese ripened for 2 and 4 months.

4. Summary of papers

Paper I

Grazing season and forage type influence goat milk composition and rennet coagulation properties

Milk protein composition and rennet coagulation properties were analysed in milk from goats grazing two types of pasture (rangeland, PR and cultivated, PC) in early (EGS) and late (LGS) grazing season. Milk from goats kept indoor and fed hay was used as comparison.

Main results

- Higher content of α_{s1}- and κ-casein was obtained in milk from goats grazing PC.
- Higher content of β-casein was obtained in milk from goats grazing PR.
- Higher milk yield, contents of total protein, casein and calcium were found in milk in EGS grazing season compared to LGS.
- Lower pH, shorter RCT, shorter firming time, and higher curd firmness were obtained in milk from EGS compared to LGS.
- Content of α_{s2}-casein, lactose and calcium were positively correlated to curd firmness

Main conclusion

The different types of forage did not influence rennet coagulation parameters; however, milk collected in late grazing season did show impaired coagulation ability. The effects observed in late season grazing may be confounded with the pre-experimental feeding treatment for the goats, as similar effects were observed in milk from goats fed hay indoor.

Paper II

Effect of forage type and season on Norwegian dairy goat milk production and quality

Milk production parameters, fatty acid composition and content of free fatty acids were analysed in milk from goats grazing two different types of pasture (rangeland, PR (woodland is the term used in the published paper) and cultivated, PC) in early (EGS) and late (LGS) grazing season. Milk from goats fed hay indoor was used as comparison.

Main results

- Milk from goats grazing PR yielded less milk, but the milk had a higher content of fat and total solids compared to PC and hay feeding.
- Content of free fatty acids did not vary with the experimental factors (feeding or grazing season).
- The milk from goats grazing PR had a lower proportion of medium-chain fatty acids C10:0–C14:0 and C18:2*c*9*t*11, and a higher proportion of C18:0, C18:2*c*9,12 and C20:0 than milk from goats grazing PC.
- Milk from grazing goats had lower proportion of the medium-chained fatty acids C12:0, C14:0 and C16:0 and higher proportion of the long-chained fatty acids C18:0, C18:1*t*11, C18:2*c*9,*t*11, C18:3*c*9,12,15, C20:0 than milk from goats fed hay.
- Content of short- and medium-chained fatty acids (C6:0–C14:0) and C16:0 were higher in late than in early grazing season, while the proportion of long chained fatty acids (C18:0, C18:1*c*9, C18:1*t*11, C18:2*c*9,12,C18:2*c*9*t*11 and C18:3c9,12,15) were lower.

Main conclusion

Milk from goats grazing rangeland produced less milk, but with a higher content of fat and total solids. Rangeland pasture did not increase the content of free fatty acids in the milk.

Paper III

Feeding a supplement rich in unsaturated fatty acids improve lipid composition and flavour in Norwegian goat milk

The objective of this experiment was to study the effect of feeding goats a concentrate supplemented with either saturated (palm oil) (SAT) or unsaturated (rapeseed oil) (UNSAT) lipids on milk fatty acid composition, content and composition of FFA, LPL-activity and flavour. A concentrate with no extra lipids was used as a control feed (CONTROL). Milk was sampled and analysed at 30, 60, 90, 120, 190 and 230 days in milk (DIM).

Main results

- Milk from goats receiving the UNSAT feed produced milk with higher content of unsaturated fatty acids. In addition, this milk had a lower content of FFA and obtained better flavour scores.
- FFA content was highly correlated with off-flavours in milk, but not to lipoprotein lipase activity.
- FFA content in milk was highest at mid-lactation, before the goats went on pasture.

Main conclusion

Feeding unsaturated lipids (rapeseed oil) had many positive effects on the milk quality, and resulted in future promises for development of concentrates based on rapeseed oil to replace the present lipid source (palm oil) used in feeds to dairy goats.

Paper IV

Effect of unsaturated lipid supplementation on milk composition and cheese making parameters

The objective was to study the effect of feeding goats a concentrate supplemented with either saturated (palm oil) or unsaturated (rapeseed oil) lipids on milk composition, rennet coagulation parameters and cheese making properties. A concentrate with no extra lipids was used as a control feed. The milk and cheeses from the three feeding groups were denoted as SAT, UNSAT and CONTROL, respectively. Milk composition was analysed six times during the lactation period (30, 60, 90, 120, 190 and 230), and cheese was made three times (90, 120 and 190) during the same lactation period including when goats were grazing mountain pasture.

Main Results

- Only minor effects of feeding different lipid supplemented concentrate on milk composition, individual casein content and rennet coagulation properties
- Lactation stage influenced all parameters except content of whey proteins
- UNSAT cheese ripened slower and had the highest moisture content and the poorest texture. SAT cheese had the highest content of free amino acids (FAA) and appeared therefore to ripen faster than UNSAT cheese.
- Cheese produced from milk at the mountain pasture (190 DIM) had a higher content of total solids and better texture than cheese produced at 90 and 120 DIM

Main conclusion

Only minor effects of feeding different lipid supplements were observed on milk protein composition and rennet coagulation properties, however, cheese composition and quality was affected. The source of lipid appeared to influence the proteolysis in cheese during ripening.

Paper V

The influence of the deletion in exon 12 of the gene encoding α_{s1}-casein (*CSN1S1*) in the milk of the Norwegian dairy goat breed on milk coagulation properties and cheese quality

The aim of this study was to investigate the effect of the deletion in exon 12 of the gene encoding α_{s1}-casein (*CSN1S1*) on milk protein composition, cheese making properties and cheese ripening. Milk from goats homozygous for the deletion (E12-00) were compared with heterozygous goats (E12-01).

Main results

- Milk from E12-00 goats had a lower content of total protein andα_{s1}-casein, and a higher content of β-casein compared to their E12-01 herdmates.
- Rennet clotting time (RCT) did not differ in milk from the two genotypes, but milk from E12-01 goats had shorter firming time (k_{20}) and obtained a firmer curd (a_{30}) than E12-01 goats.
- Cheese made of milk from E12-00 goats had a higher moisture content and more pronounced rancid flavour compared to E12-01.

Main conclusion

Milk from goats heterozygous for the deletion in exon 12 (E12-01) was more suitable for cheese production, because the cheese obtained a better texture and flavour score than milk from homozygous goats (E12-00).

5. Key results and general discussion

5.1 Goats on pastures - effects in milk

Rangeland and mountain pastures are important feed resources during the summer months, and mountain farming has long traditions in Norway. Inferred quality with regard to coagulation properties and especially off-flavours [71; 72] has been reported in milk during the grazing period. Decrease in the body weight due to decreased forage quality and energy spent on wandering on rangeland pasture is believed to increase content of FFA and subsequent off-flavours in the milk [108; 109]. However, the physiological mechanisms still remains unclear as another study have shown that higher energy intake and positive energy balance were correlated to high levels of FFA [110; 111]. Moreover, it has been shown that milk composition varies during the lactation cycle [23] and LPL-activity is at maximum in mid-lactation [61]. In contrast to cow-dairying, where calving proceeds all year around, goats usually have their kids during springtime. Therefore, compositional factors determined by lactation stage and by season (time when goats are let on pasture) may be confounded.

In Paper I and II, the effects of forage type on goat milk composition and production parameters and were studied. One group of goats grazed rangeland (PR) and another group grazed cultivated pasture (PC). Two groups of goats were indoor receiving hay of high (HH) or low (HL) quality, respectively. The experiment was conducted twice during a grazing season: in July (early grazing season, EGS) and in mid-August-September (late grazing season, LGS).

Type of forage affected milk yield and composition. Goats on PR yielded less milk (27 %), but had a higher fat content than goats on PC. As Goats on PR had a higher feed intake, the lower milk yield is most likely explained by more energy spent on wandering and not by the quality of the forage on PR (Paper II). Milk from pastoral goats had higher protein and casein content compared to hay fed goats. The casein composition was different in pastoral compared to hay fed goats, and by grazing PR vs PC (Paper I). The most prominent effect of PC was on α_{s1}-casein (Figure 11), but κ-casein levels were also higher. The increase in α_{s1}-casein and κ-casein were observed in both grazing seasons. Milk from goats on PR yielded more β-casein in both EGS and LGS (Paper I and Paper II).

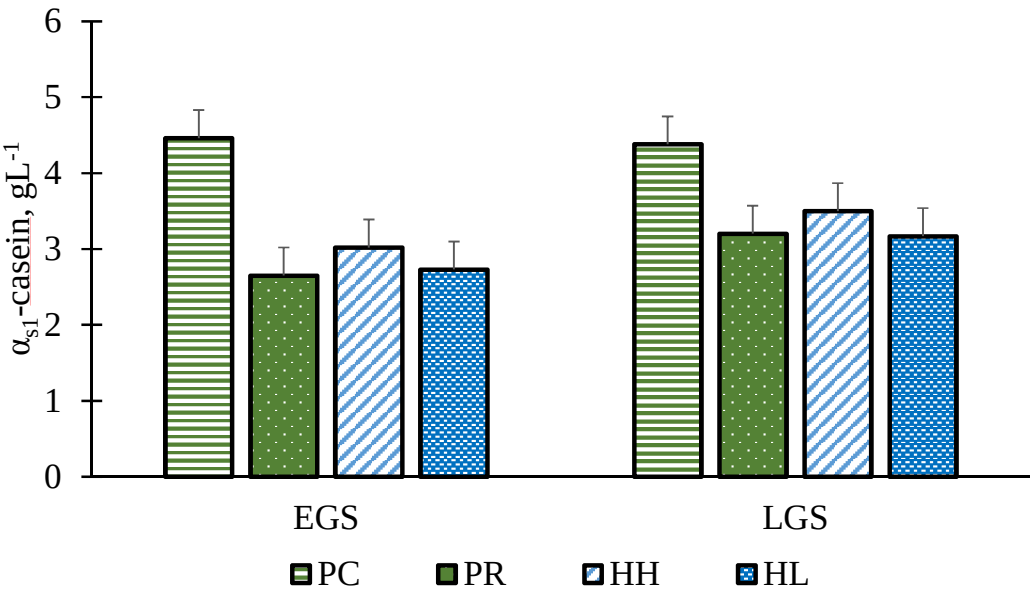

Figure 11. Content of α_{s1}-casein in Early (EGS) and Late (LGS) grazing season. Two types of pasture (green); cultivated (PC) and rangeland (PR) were compared to two types of hay (blue); high (HH) and low (HL) quality. Modified figure from Paper I [(107)].

One would probably expect improved rennet coagulation properties when the content of α_{s1}- and κ-casein increased. However, apart from longer RCT in milk from goats grazing, no differences in rennet coagulation parameters were found between the different forage types. The grazing season influenced the rennet coagulation parameters: weaker curd (a_{30}) and especially longer firming time (k_{20}) occurred in LGS. A large proportion of the milk samples, which included all E12-00 goats, did not obtain k_{20} in LGS. The explanation for the impaired rennet coagulation properties in LGS remains unclear, as this was also observed for the control groups receiving hay indoor. The effect of season was confounded with the pre-experimental period because the goats in LGS was grazing on rangeland before they were allocated to their feeding groups, while the goats in EGS received silage in the pre-experimental period (Figure 9). A plausible explanation of the changes that occurred from EGS to LGS may be attributed to increased plasmin activity. Factors like stress and late lactation are known to increase the plasmin activity in milk [(112; 113)], however, the goats were in the same lactation stage in both EGS and LGS. Degradation of caseins by plasmin may impair the curd firmness and reduce the cheese yield [(114)]. In addition, the milk yield was reduced in LGS. Plasmin has been reported to generate a peptide from β-casein (fragment 1-28) that blocks the K^+ channels of the mammary epithelial cells, which is associated with

reduced milk yield [113]. The reduction of α_{s2}- and β-casein content in LGS may imply degradation of these caseins by plasmin, which is known to impair the clotting properties of milk [112; 115]. Impaired rennet coagulation properties in milk from E12-00 goats were observed at mountain pasture in a later study (Figure 12, Inglingstad, unpublished data). The E12-01 goats displayed even better rennet coagulation properties at mountain pasture (190 DIM) than at early lactation stages (Paper V), and the difference between the two genotypes were prominent indeed (Figure 10).

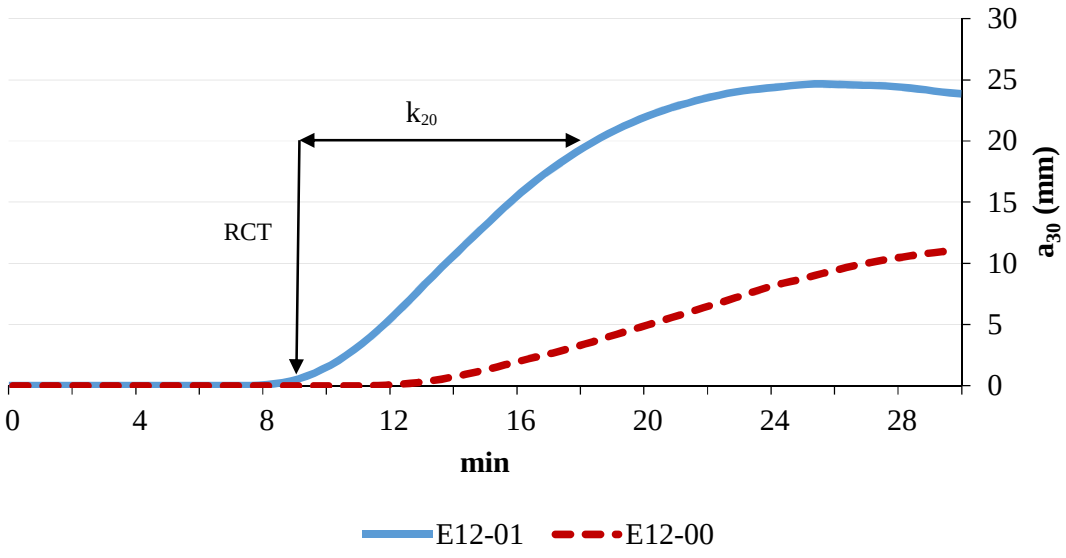

Figure 12. Rennet coagulation parameters measured at mountain pasture in milk from goats with different genotype at exon 12 CSN1S1. E12-01 (n=21) and E12-00 (n=10). Unpublished results.

In a previous study [108] the level of FFA in milk increased during the mountain grazing period, therefore a higher content of FFA was expected on PR, especially in LGS due to decreasing pasture quality in the later season [17]. However, no effect of forage nor grazing season was found for levels of FFA in milk (Paper II). In fact, the levels of FFA were lower in EGS than in LGS. In another study (Paper III), the FFA level peaked around 90 DIM when the goats were still indoor receiving silage. The level of FFA did not increase in milk at the end of the mountain grazing period (190 DIM). This implies that increased level of FFA may be caused

by factors related to lactation stage rather than energy spent on wandering or the decrease in pasture quality.

5.2 Lipid supplements – effects in milk

Fatty acid composition in milk can be altered by feeding lipid supplements [116; 117], however, excess of especially unsaturated lipids may cause milk fat depression in cows [118]. Goats responds differently to lipid feeding, and it is possible both to increase the milk fat content and change the lipid composition [61]. In addition, French studies have shown that by feeding unsaturated lipids to goats, the LPL-activity decreased [119]. However, in Norwegian goats the frequency of off-flavors was reduced when receiving extra saturated lipids (mainly C16:0 and C18:0) [71]. Based on the latter study, the hypothesis of the mechanism for the improved flavor was that increased cholesterol levels would provide increased stability to the MFGM and thereby reducing the LPL's access to the triglycerides. This would lead to lower content of FFA and hence reduce the off-flavours [71]. The type of fat used in the study described above is now a standard fat source in concentrate to dairy animals [120], as calcium soaps of palm oil. However, lately the use of palm oil in food, and now also in feed, has been severely criticized [121]. The demand for more sustainable lipid source of both food and feed is increasing. Rapeseeds is the only type of oil seed that is produced in significant amounts in Norway. Rapeseed oil (UNSAT) was therefore included as an experimental comparison to palm oil (SAT), also because of the positive effects observed of unsaturated lipids in other studies [119]. In addition, concentrate with no extra fat was included as a control (CONTROL). The three different concentrates were examined for the effect on milk composition, milk flavor and cheese making properties.

As expected, the fat content in milk increased when the goats received concentrate supplemented with extra fat (Paper III). This is also shown by several others [122-124]. A higher fat content normally increases the total solids (TS) and the cheese yield [125]. In addition, fat content is positively correlated to the mean size of MFG in goats (Paper III) [74], and cows [126] and buffalos [127; 128]. The lipid source of the goats feed influences the lipid composition of the milk (Paper III) [61], and therefore increasing the proportion of unsaturated fatty acids is possible. An increased intake of unsaturated fat and a decreased intake of saturated fat in the human diet is recommended by health authorities worldwide [129; 130]. Pasture (Paper II) and rapeseed oil (Paper III) both increased the proportion of unsaturated lipids at the expense of the saturated fatty acids in the milk, and this is also supported by several other studies [131;

[132]. When no extra fat was fed to the goats, the proportion of saturated fatty acids with less than 16 carbons and odd- and branched chained fatty acids increased. The odd- and branched chain fatty acids originates from ruminal metabolism of branched-chain amino acids, propionate and butyrate [61]. The SAT concentrate increased the content of C16:0 and C16:1 which reflects the high content of C16:0 in the SAT concentrate, and this is in accordance with a previous study by Eknæs et al. [72]. This study showed that when the content of C16:0 increased in the milk, the frequency of tart and rancid flavors were reduced, but without affecting the content of FFA. In the present study (Paper III), the UNSAT milk had significantly lower content of FFA at mid lactation (90-120 DIM) compared to SAT and CONTROL milk. This was somewhat unexpected, as we hypothesized that feeding more C16:0 would increase the cholesterol levels in the MFGM and thereby increase the stability towards lipolysis.

The highest levels of FFA were measured when the goats were in mid lactation (90-120 DIM) and fed silage Previous studies showed that the LPL-activity in French goat milk was highest in mid lactation [61] and decreased if unsaturated lipids were fed to the goats [116; 133]. Similar trends of lactation stage and UNSAT diet were observed in our study (Paper III), however, the effects were not as prominent as in the French studies, and not directly correlated to content of FFA (r= -0.2, n=186). Therefore, there must me other factors involved that explains the elevated levels of FFA in some goats and this warrants further research. While content of FFA and LPL-activity were not correlated, there was a strong negative correlation (r= -0.8, $P<0.0001$, n=184) between high levels of FFA and good milk flavour. UNSAT milk received best score for its flavour, while flavour scores of SAT and CONTROL milk did not differ.

The inclusion of rapeseed in the goats' diet is promising with regard to both an improved fatty acid composition, reduction of content of FFA and improved flavour. However, effects of replacing saturated with an unsaturated lipid source warrants investigations of the non-fat components of milk and of its technological properties. Until today, very little data covering these issues is published. Therefore, in Paper IV, we investigated the effect of SAT, UNSAT and CONTROL concentrates on milk content of non-fat components and rennet coagulation properties during a whole lactation cycle. In addition, cheese was made of bulk milk from the three feeding groups at three different lactation stages (90, 120 and 190 DIM) [134]. The milk parameters that was investigated was total contents of protein, casein, individual caseins, and lactose, SCC and pH, in addition to rennet coagulation parameters. Our investigation revealed that these parameters were not significantly influenced by the lipid source of the concentrate.

In this study, an experimental cheese of Havarti type was produced. Cheese analysis were performed in fresh cheese, and after 2 and 4 months of ripening. The content of free amino acids (FAA) reflects proteolytic activity and may therefore be used as a measure of the degree of ripening [135]. SAT cheese had a higher content of FAA compared to UNSAT, and appeared therefore to have higher proteolytic activity. UNSAT cheese received lower scores for texture and was described as more doughy. As there was no major differences in the protein composition of the milk that can explain this difference, it is likely that the observed differences are due to different fatty acid composition or content of FFA. As at least the short chain FFA are relatively polar, they are probably gone with the whey at drainage. Hence, it is likely that the fatty acid composition explains the differences between the cheeses. UNSAT milk had a higher proportion of unsaturated lipids (Paper III), and from other studies it is known that the fatty acid composition in milk and the corresponding cheese is very similar [136]. A higher content of unsaturated fatty acids gives a softer and more spreadable cheese, but may also be characterized as doughy with poor texture compared the cheeses with a higher proportion of saturated fatty acids (Paper IV). The best texture was obtained for cheeses made at 190 DIM of milk from goats grazing mountain pasture, and this was probably related to increased content of total solids in the cheese. In addition, curd firmness of the rennet coagulated milk was higher at 190 DIM compared to 90 and 120 DIM.

5.3 Polymorphism at the α_{s1}-casein locus- effects in milk

Milk from goats homozygous for the deletion in exon 12 (E12-00) has previously showed to have a lower content of protein, fat and lactose, but higher frequency of elevated levels of FFA and off-flavours [79]. Moreover, E12-00 goats often displays impaired rennet coagulation properties [81]. It was suggested to use genetic selection of casein alleles already in 1995, when this genotype first was discovered [75]. The Norwegian researcher of this study, S. Lien, suggested an investigation of the cheese making properties ahead of the selection. However, this investigation was not undertaken until 2012 [134] and was included as a part of Paper V. Genetic testing and selection based of this allele was not implemented in the breeding program of Norwegian Dairy goats before 2008 [15]. This allele was originally described as an 0-allele [75], because there was no evidence of α_{s1}-casein on protein level performed by IEF gel electrophoresis [78]. Therefore, it was surprising to find a protein eluting at the same time as α_{s1}-casein in all E12-00 goat milk samples using capillary zone electrophoresis (Paper I, Paper IV and Paper V), even if the levels were low. If the peak corresponding to α_{s1}-casein in these goats truly is an evidence of this protein, remains to be investigated. While the rennet

coagulation properties have been investigated [81], the properties of cheeses made from milk from E12-00 goats were unknown. Therefore, the aim of Paper V was to study the cheese making properties and cheese quality during ripening in milk from E12-00 goats compared to E12-01 goats. The E12-00 cheese was more doughy with a poorer texture as compared to E12-01 cheese, and this is probably explained by lower contents of total solids. Moreover, E12-00 milk had a higher content of β-casein and larger casein micelles [81] (Paper I), which may explain the more hydrated cheese matrix. The composition of FAA did also differ in cheese from E12-00 and E12-01 goats, which indicates different proteolytic activity. The cheesemaking was performed at 30 and 60 DIM. Even though the content of FFA in milk was low at this lactation stage, and with no difference between E12-00 and E12-01, the cheeses from E12-00 developed a pronounced rancid flavour during ripening (Paper V).

5.4 Factors influencing the rennet coagulation properties in Norwegian goat milk

To increase the understanding of whether rennet coagulation properties were influenced by management or genetic factors, 25 goats with poor rennet coagulation properties from the north of Norway were moved to the university farm (Ås) in 1990 [137; 138]. These goats were compared to goats at the university farm over two subsequent lactations, in addition, the performance of the progeny from two groups of goats was compared. While content of milk components (fat, proteins, lactose and total solids) seemed to be inherited, the rennet coagulation properties seemed influenced by both management and genetic factors [139].

Therefore, in this study effects of different forage (Pasture or hay, Paper I) and lipid supplementation (rapeseed or palm oil, Paper IV) on rennet coagulation properties were investigated. Feeding did not seem to affect rennet coagulation parameters directly (Paper 1 and Paper IV), however in Paper 1 an effect of grazing season, with impaired rennet coagulation in late season, was observed. This effect was most likely explained by the pre-experimental treatment. In another experiment, the rennet coagulation properties obtained when the goats were grazing mountain pasture was different between E12-01 and E12-00 goats (Figure 12). Therefore, it seems like the rennet coagulation properties are impaired in some goats after a period with reduced forage quality and more energy spent on wandering, and the E12-00 goats seems more susceptible (Paper 1 and unpublished data).

Content of α_{s1}-casein is positively correlated to curd firmness (a_{30}) [140; 141], which is confirmed in this study (Paper I, Paper V and unpublished data (Figure 13). However in Paper 1, content of α_{s2}-casein was stronger correlated than α_{s1}-casein to a_{30}. This strong correlation

was not confirmed in a later study (unpublished data), however, if only E12-00 goats are concerned, positive correlations ($r > 0.47$, $P<0.001$) between both α_{s2}-, β- and κ-casein are found (unpublished data). The factors most important to curd firmness appears to be total casein, total protein and content of α_{s1}-casein [140] (Figure 13). Determination of rennet coagulation by use of the spectra obtained by the milk routine analysis (Fourier Transform Infrared analysis, FTIR) would be useful both with regards to payment, but also with regards to genetic selection as the many parts of this spectra are inheritable [142; 143]. Some preliminary results show promising possibility for prediction of content of total casein, α_{s1}-casein and curd firmness [144], however, further development is required.

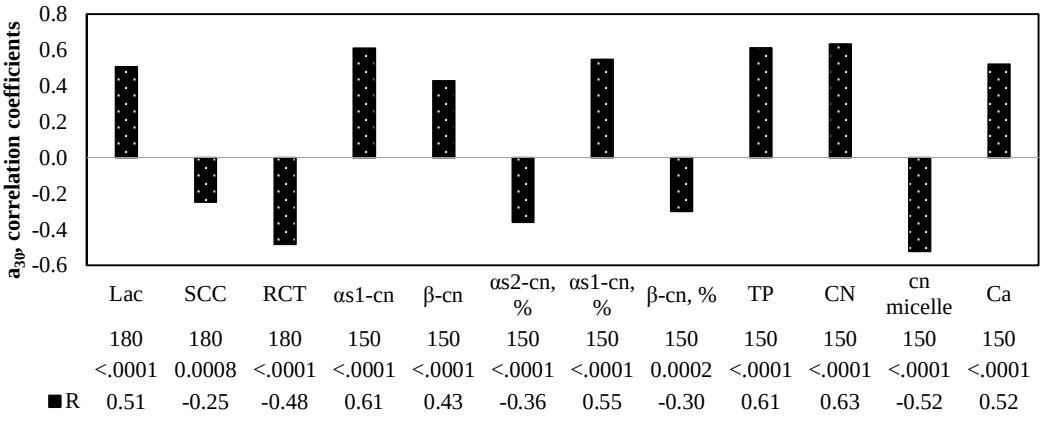

Figure 13. Pearson correlation coefficients of some selected parameters and a_{30}. Number of observations and P-values are indicated. (Lac=lactose, %, SCC=log10 ml somatic cell count, RCT, rennet clotting time, min, cn=casein, TP= total protein, Ca= calcium Unpublished data.

5.5 Factors influencing the level of FFA in Norwegian goat milk

The body condition of the goat has been one factor to explain or understand why some goats displays elevated levels of FFA in their milk in certain periods (Paper II), [108; 110; 111], however, the results of these studies are ambiguous and a clear conclusion can hardly be drawn. As indicated earlier in this thesis, the correlation of LPL-activity and FFA is low. Therefore, one should probably investigate the *milk* rather than the animal to attempt to identify the direct cause of the elevated lipolysis rate. When these factors are identified in the milk, it will probably be easier to relate them to attributes of the animal itself, and whether the reason is

genetic or physiologic or a combination of these factors. The MFGM protects the triglycerides from lipolysis by the LPL. It is known that disruption of MFGM by e.g. homogenisation and agitation can cause extensive lipolysis [145]. All milk samples in the three main experiments in this thesis (A, B and C) were handled with great care using a standardized protocol. Still the variation in content of FFA between samples was large. To increase the understanding of why some milk samples have a high content of FFA whereas other samples have a low content, a few milk samples were analysed for their phospholipid composition and cholesterol content (Paper III). Samples with elevated levels of FFA had a higher content of cholesterol and lysolecitin (a result of phospholipase activity [63]) and a lower content of phosphatidyletanolamine than samples with low levels of FFA. Unfortunately, only six samples were analysed, which is too few to draw any conclusion. Nevertheless, the results strongly indicate that the composition of the MFGM may be of great importance, and other studies [71; 72] pointing to the MFGM composition as an important factor. Cholesterol, together with sphingomyelin, are the major constituents of lipid rafts in MFGM; structures involved in different cellular processes [146], and cholesterol is reported to affect the MFGM organization in bovine milk [147].

It has been shown that when cows received a diet supplemented with unsaturated lipids, the concentration of unsaturated lipids increased in the MFGM [65]. Therefore, it is likely that the goats receiving unsaturated lipids in present study (Paper III) also increased their content of unsaturated lipids in the MFGM. Moreover, these goats did also have the lowest content of FFA in their milk. Increased fluidity of the MFGM may therefore increase the resistance against lipolysis of the triglycerides.

Based on the findings in this thesis, it appears that FFA levels in the milk are highest in mid lactation and from E12-00 goats. Furthermore, levels of FFA decrease if rapeseed oil is fed to the goats. There is no evidence for increased levels of FFA on mountain or rangeland pasture. As the frequency of the defective allele in *CSN1S1* used to be extremely high, it is likely that most of the goats in the previous studies [71; 72; 109] were E12-00. The response to energy intake and selection of feeds are different between goats with strong and weak alleles [148; 149], which may partly explain the contradictory findings from earlier studies in the nineties and turn of the century in Norwegian goats [71; 72; 109] compared to the findings in these present studies.

6. Current status, concluding remarks and further perspectives

The results of studies on feeding, breeding and milk quality has been successfully implemented in the goat milk production during the last fifteen years. The implementation of genotype testing in the breeding programme has reduced the frequency of E12-00 goats in the population. In addition, the "Healthier goats" project has led to increased milk yield and quality and improved the health and welfare of the goats. The milk quality has increased thanks to a joint effort of researchers, advisors and last, but surly not least: the producers. They really deserves honour for their willingness to apply and implement the findings of scientific research in practice. Now the TINE dairy has to come forward and follow up in the development of new product and marketing. The milk is surly too good to be fed pigs or poured into the drain! The project "Quality milk for cheese production" has increased the knowledge of breeding, pasture quality, feeding and physiology and how these parameters influences the milk quality. The focus of this thesis was milk quality and milk composition, and the main conclusions are:

- Pasture did not increase the content of FFA. Elevated levels of FFA occurred at mid-lactation.
- Rapeseed oil decreased the level of FFA at mid-lactation.
- There is a strong correlation between a high level of FFA and off-flavours.
- Lipid supplemented concentrate affects the fatty acid composition, but not the protein composition.
- Lactation stage, genotype and grazing season affects protein composition and rennet coagulation properties.
- Cheese made of milk from E12-01 goats was preferred over cheese made from milk of E12-00 goats

Because of the positive effects of rapeseed oil on FFA and flavour, rapeseed oil should be implemented in commercial feed for dairy goats. The composition of the MFGM should be investigated to understand the mechanism of why some goats are more prone to have elevated levels of FFA in their milk. There is still a great potential of increasing the rennet coagulation properties of the Norwegian goat milk. By identifying SNPs or areas in the FTIR spectra (Milkoscan) correlated to curd firmness and/or content of α_{s1}-casein, genetic selection for improved rennet coagulation properties would be possible. With such a good goat milk quality, further development of products and increased marketing is crucial.

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8. Enclosed papers I-V

Paper I

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Grazing season and forage type influence goat milk composition and rennet coagulation properties

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ABSTRACT

Two different types of pasture (cultivated and rangeland) and 2 different hay qualities (high and low quality) were examined for their effects on goat milk composition and rennet coagulation properties. Furthermore, the effect of dietary treatments in both the early and late grazing season was studied. As lactation stage is known to influence milk composition, the goats in the early and late grazing season were in the same lactation stage at the start of the experiment. The milk composition was influenced both by dietary treatment and season. Milk from goats on pasture was superior to those on hay by containing a higher content of protein and casein, and the goats on cultivated pasture had the highest milk yield. Casein composition was significantly influenced by forage treatment. Goats grazing on cultivated pasture had higher contents of α_{s1}-casein and also of κ-casein compared with the other treatments, whereas goats grazing on rangeland had the highest content of β-casein. Factors such as milk yield, casein micelle size, α_{s2}-casein, and calcium content were reduced in late compared with early season. More favorable rennet coagulation properties were achieved in milk from the early grazing season, with shorter firming time and higher curd firmness compared with milk from the late grazing season, but the firming time and curd firmness were not prominently influenced by forage treatment. The content of α_{s2}-casein and calcium in the milk affected the firming time and the curd firmness positively. The influence of season and forage treatment on especially milk yield, casein content, and rennet coagulation properties is of economic importance for both the dairy industry and goat milk farmers.

Key words: goat milk, milk rennet coagulation properties, individual casein composition, pasture

INTRODUCTION

Norway has a long tradition of goat milk production. Most of the milk from about 40,000 dairy goats is used for production of the traditional brown whey cheese (Brunost). The demand for brown whey cheese among Norwegian consumers is declining, and the interest in rennet- and acid-coagulated cheeses is increasing among the dairy industry and consumers. However, production of these cheeses requires a milk of a more stable and higher quality than milk used for brown whey cheese. Quality cheese milk has high DM content (casein and fat is most important), low SCC, and low susceptibility to excessive lipolysis, maintaining a low content of FFA. In addition, the ability to clot by the action of rennet and achievement of a firm curd are important factors in cheese manufacture (Skeie, 2010).

Previous studies have shown that the population of Norwegian goats has a high frequency of animals with low or no synthesis of α_{s1}-CN (Devold et al., 2011). This is caused by an extremely high frequency (0.73) of a defective allele with a single nucleotide deletion in exon 12 of the gene encoding α_{s1}-CN (*CSN1S1*; Hayes et al., 2006; Dagnachew et al., 2011). Until now, Norwegian dairy goats are the only breed known to carry this deletion. In addition to low or no expression of α_{s1}-CN in the milk, this deletion correlates with a reduced content of protein, fat, and lactose; a high content of FFA; and tart and rancid flavor (Dagnachew et al., 2011). Milk from Norwegian goats shows poor rennet coagulation properties and sensory quality in periods of the year. The milk-quality challenges are more pronounced during the grazing season (Eknæs and Skeie, 2006). Traditionally, goat milk production is seasonal, with kidding in winter and early spring and with peak milk production during the summer grazing season. During the grazing season, the goats graze to a large extent on natural unimproved grassland or free range in forest and mountain grasslands. The quality of these pastures is variable and declines with forage production during the grazing season (Lunnan and Todnem, 2011). The

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decline in forage and milk quality coincides with advanced lactation stage, another factor associated with increased FFA content in goat milk (Chilliard et al., 2003). Thus, the effects of the grazing season and the lactation stage on milk quality may be confounded. The high degree of polymorphism at the α_{s1}-CN locus (for review, see Marletta et al., 2007) is known to affect both casein content and rennet coagulation properties (Clark and Sherbon, 2000; Devold et al., 2011). However, few studies exist focusing on the effect of forage quality on rennet coagulation properties and protein quality (i.e., the casein content and composition of individual caseins in milk from dairy goats).

Hence, the objective of this work was to study the effects of grazing season (early and late), forage type [hay (high- and low-quality hay)], and pasture (cultivated and rangeland) on milk composition and rennet coagulation properties. To separate the effects of these factors from the effect of lactation, all goats were in the same lactation stage at the start of the feeding experiment.

MATERIALS AND METHODS

Experimental Design and Diets

The experiment comprised 80 Norwegian dairy goats, located at Gibostad research farm, Norway (69°21.397′N, 17°56.319E). A simplified overview of the experimental design is shown in Figure 1. The goats were genotyped according to Hayes et al. (2006) with respect to the deletion in exon 12 the gene encoding α_{s1}-CN (*CSN1S1*). The individuals were blocked according to lactation number (5 groups: 1 = first lactation, 2 = second lactation, 3 = third lactation, 4 = fourth lactation, and 5 = more than 4 lactations) and genotype [homozygous (**E12-00**) or nonhomozygous (**E12-01/E12-11**) for the deletion in exon 12], and the goats within each of these blocks were further randomly divided into 2 groups [early (**EGS**) and late (**LGS**) grazing season]. The goats in EGS were mated approximately 8 wk before those in LGS, and the average kidding date was February 2 (SD = 9 d) and April 1 (SD = 12 d) for the EGS and LGS group, respectively. From kidding until the start of the grazing season (June 28, 2010) the goats received the same diet: silage fed ad libitum and 1.1 kg of concentrate/d per goat. The concentrate was produced for this experiment by Felleskjøpet Agri (Storsteinnes, Norway) and was a mixture with the following ingredients (g/kg): barley (278), oat (263), wheat bran (159), sugar cane molasses (65), sugar beet pulp (60), extracted soybean meal (46), oil seed (41), SoyPass (Denofa AS, Fredrikstad, Norway, WI; 34), limestone (19), and other minerals and vitamins (35).

At approximately 130 DIM (June 28 and August 16 for the EGS and LGS group, respectively), the goats were randomly assigned to 4 homogenous treatment groups. The treatment groups were balanced for genotype and lactation number and each consisted of 10 goats. The 4 treatments were cultivated pasture (**PC**), rangeland pasture (**PR**), high-quality hay (**HH**), and low-quality hay (**HL**). The chemical composition for the 2 hay qualities (HH and HL) and concentrate used in the experiment and of the silage fed indoors from kidding to the start of the grazing season are given in Table 1. The goats on hay were kept indoors in pens and the hay was fed ad libitum, allowing 10% refusals. The goats on pasture grazed day and night. The PC was a ley (2.1 ha in area) in its first production year dominated by *Phleum pratense* and *Festuca pratensis*. The daily allowance was on average 13 kg of DM/goat. The PR was approximately 300 ha with the following vegetation types (% of land area): blueberry-birch woodland (41), fen (22), meadow-birch forest (21), lichen/heather-birch woodland (6), wet woodland (4), natural grassland (4), and spruce woodland (2). Dominating species were birch (*Betula pubescens*), bilberries (*Vaccinium myrtillus*), Swedish cornel (*Cornus suecica*), wavy hair-grass (*Avenella flexuosa*), and sweet vernal grass (*Anthoxanthum odoratum*).

The LGS group grazed together with the PR group of the EGS goats until August 16, 2010. The forage treatment periods lasted for 3 wk. All goats during the forage treatment period were supplemented with the same concentrate mixture as fed during the indoor period at a rate of 0.9 kg/d and the concentrate was given during milking twice daily.

To summarize, the design applied was a 2 × 4 factorial with season (EGS and LGS) as one factor and forage type (PC, PR, HH, and HL) as the other factor. The 10 goats in each treatment (PC, PR, HH, and HL) were randomly divided into 2 groups (pens) with 5 goats, accounting for genotype and lactation number. In the 2 hay treatments, the 5 goats in each group within treatment were kept indoors in 2 separate pens, whereas the goats within each pasture treatment grazed together. The procedures in the experiment were according to the regulations set by the Norwegian Animal Research Authority (Oslo, Norway).

Feed Intake, Feed Sampling, and Analysis

Feed intake on pasture was estimated by the use of the *n*-alkane technique (Mayes et al., 1986) and is reported for the current experiment by Steinshamn et al. (2014). Procedures for sampling and preparation of samples of grazed plants, hay, and concentrate are also described by Steinshamn et al. (2014). The chemical composition

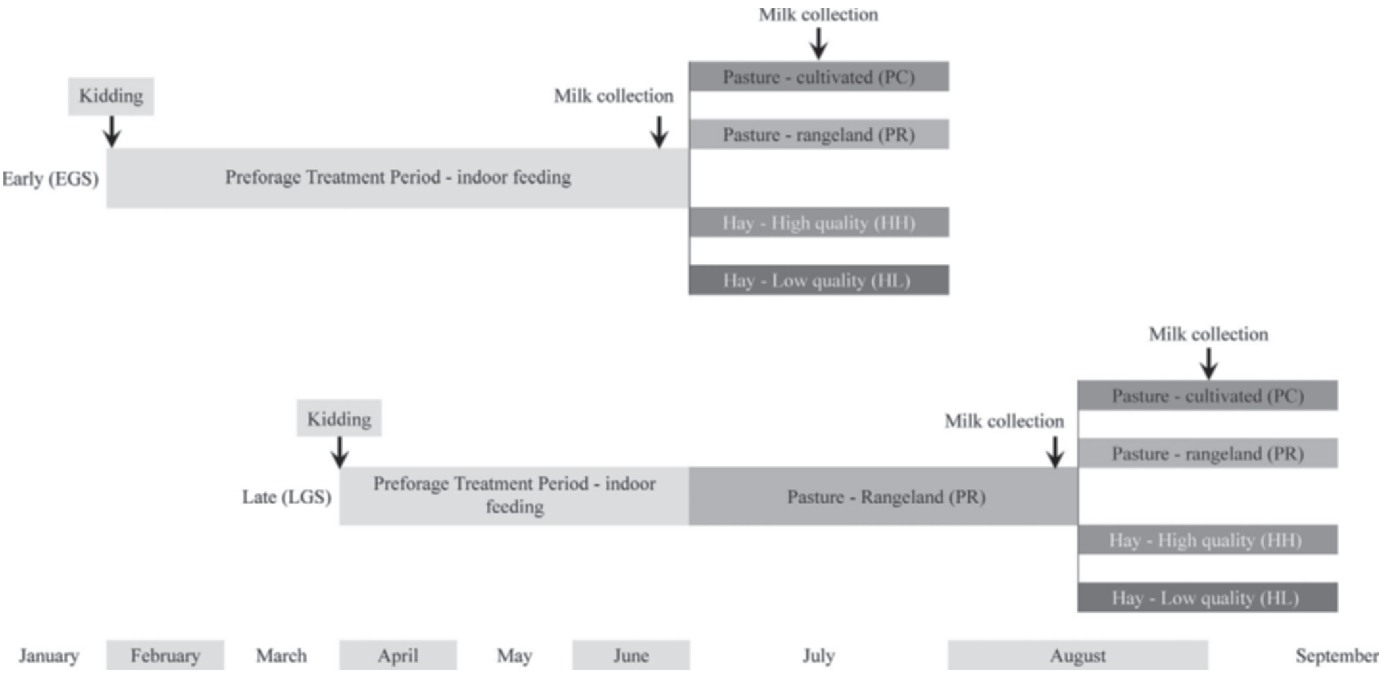

Figure 1. Experimental design. Eighty goats were divided into 2 groups: early (EGS) and late (LGS) grazing season, with 8 wk difference in kidding time. At 130 DIM, they were further allocated into 4 dietary treatment groups: low-quality hay (HL), high-quality hay (HH), rangeland pasture (PR), and cultivated pasture (PC). The arrows indicate when milk yield was measured and milk samples for chemical analysis were collected.

of feed and pasture samples were analyzed at the Dairy One Inc. Forage Testing Laboratory (Ithaca, NY) with wet chemical procedures. Crude protein content was determined using AOAC method 990.03 and crude fat by AOAC method 2003.05 (AOAC, 1990). Heat-stable, α-amylase-treated, sodium sulfite NDF was determined using an Ankom fiber analyzer (Ankom Technology Corp., Fairport, NY) based on procedures described by Van Soest et al. (1991). Digestibility of DM and NDF was determined in vitro after incubation for 48 h using the Ankom DaisyII filter bag technique (Ankom Technology Corp., Macedon, NY). The NE_L 3× maintenance was predicted from total digestible nutrients according to the NRC (2001). Daily intake of hay per goat per pen was calculated as kilograms of DM offered per pen − kilograms of DM refused per pen divided by the number of goats per pen. Feed quality of consumed hay was calculated as the difference between quantity of the quality parameter offered (e.g., CP) and quantity of the quality parameter refused divided by the DM consumed. The quality of the herbage consumed on pasture was calculated as the weighted mean of the nutritive value of each botanical component of the diet. Least-squares optimization using available alkane and alcohols was used to obtain estimates of the botanical composition of the diet. The feed quality of the forages as consumed and of concentrate is presented in Table 1.

Milk Sampling and Analysis of Milk Composition

Milk samples were collected twice during each grazing period (EGS and LGS): 1 wk before the goats were allocated to the forage treatments (preforage treatment period) and 2 wk after onset of the dietary treatment (forage experimental period; Figure 1). The goats were milked twice daily (0630 and 1600 h) and milk yield recorded. Samples from 4 subsequent milkings (2 d) were pooled to have 1 milk sample from each goat. Samples for analysis of casein composition and minerals were stored at −20°C, and samples for analysis of casein micelle size (preserved with sodium azide) were stored in room temperature (1 night), whereas the rest of the samples were stored at 4°C until analysis.

Milk Composition (Routine Analysis). Milk samples were preserved with bronopol (2-bromo-2-nitropropane-1,3-diol; D & F Control Systems Inc., San Ramon, CA) and analyzed for fat, protein, lactose, urea, and SCC by Fourier-transform infrared spectroscopy (MilkoScan CombiFoss 6500; Foss, Hillerød, Denmark).

Milk Fat Removal. Before analysis of protein and casein micelle size, the milk was skimmed. The samples were centrifuged at 2,000 × g for 20 min at 25°C and then the milk fat was crystallized at −20°C for 20 min before the milk fat was removed by a spatula.

Table 1. Chemical composition (g/kg of DM, with SD in parentheses) of the forages, concentrate, and silage fed indoors preexperimentally

Item	Pasture type[1]				Hay quality[2]		Silage (n = 9)	Concentrate (n = 6)
	PC-EGS (n = 2)	PR-EGS (n = 2)	PC-LGS (n = 2)	PR-LGS (n = 2)	HH (n = 4)	HL (n = 4)		
NE_L (MJ/kg of DM)	7.19 (0.01)	6.97 (0.03)	6.65 (0.01)	6.46 (0.07)	5.57 (0.01)	4.66 (0.01)	5.9 (0.46)	7.5 (0.10)
CP (g/kg of DM)	175 (2.6)	138 (5.3)	171 (0.03)	104 (2.5)	198 (0.3)	112 (1.3)	138 (18.2)	191 (2.8)
NDF (g/kg of DM)	368 (4.6)	356 (8.2)	411 (1.3)	356 (15.5)	562 (0.2)	642 (0.3)	539 (49.4)	256 (27.8)
Crude fat (g/kg of DM)	44 (0.7)	46 (3.3)	34 (0.02)	44 (1.1)	29 (0.1)	21 (0.3)	42 (11.0)	51 (3.6)

[1]PC = cultivated pasture; PR = rangeland pasture; EGS = early grazing season; LGS = late grazing season.
[2]HH = high-quality hay; HL = low-quality hay.

Total Protein, Noncasein Protein, and NPN. Total nitrogen (TN), noncasein nitrogen (**NCN**), and NPN contents of skim milk samples were measured by a Kjeltec 8400 analyzer (Foss) according to Aschaffenburg and Drewry (1959) modified by Devold et al. (2011). The NCN fraction was prepared from 2 mL of milk and the casein fraction was precipitated at pH 4.2 instead of pH 4.6 using a buffer of 1 *M* acetic acid and 1 *M* sodium acetate [37:3; buffers prepared according to Pearse (1980)]. All samples were run in triplicate. The calculations used for total protein (**TP**) percentage and casein percentage were as follows:

$$\text{Total protein (TP)} = (\text{TN} - \text{NPN}) \times 6.38;$$

$$\text{Whey protein (WP)} = (\text{NCN} - \text{NPN}) \times 6.38;$$

$$\text{Casein} = \text{TP} - \text{WP}.$$

Quantification of Individual Caseins. To quantify individual caseins, a combined approach of cation-exchange chromatography and capillary zone electrophoresis was followed. Cation-exchange chromatography was implemented to obtain protein standards from the isoelectric casein of a genotyped goat that was homozygous for strong alleles at α_{s1}-, α_{s2}-, β-, and κ-CN. Cation-exchange chromatography was performed on an ÄKTA purifier (GE Healthcare, Freiburg, Germany) equipped with a HiLoad 26/10 SP Sepharose HP column (GE Healthcare, Uppsala, Sweden). The analysis was performed according to Gómez-Ruiz et al. (2004) with the following modifications: the 4 caseins were obtained by a unique separation with buffer A [6 *M* urea, 0.02 *M* Na acetate, and 64 μ*M* dithiothreitol (DTT)] adjusted to pH 4 and buffer B, which had the same composition and pH, but with the addition of 1 *M* NaCl. Casein fractions were eluted with the following gradient: 0% B for 0.33 of the column volume (**CVol**), 2.5% B for 0.02 CVol, 7.5% B for 1.4 CVol, 16% B for 1 CVol, 18% B for 3.5 CVol, 32% B for 1.25 CVol, 32% B for 1.5 CVol, and 40% B for 2 CVol. The flow rate was 5 mL/min and the absorbance was recorded at 280 nm. Peaks of individual caseins were collected by a fraction collector and lyophilized after dialysis.

Capillary electrophoresis analysis was performed by use of a Beckman P/ACE MDQ system controlled by 32 Karat Software (version 8.0; Beckman Instruments Inc., Fullerton, CA). The instrument was equipped with a UV detector set at 214 nm. Casein separation was achieved according to the method described by Valenti et al. (2012). Calibration curves for quantification of the individual caseins were performed, taking into account the expected range of variation for each casein fraction (Gómez-Ruiz et al., 2004).

pH. The pH of milk (20°C) was measured using a pH meter (PHM 61; Radiometer, Copenhagen, Denmark) coupled to a pH electrode (pHC2005; Radiometer Analytical SAS, Villeurbanne Cedex, France).

Mean Size of Casein Micelles. The mean size of casein micelles was measured by photon correlation spectroscopy using the Zetasizer 3000 HS particle size analyzer (Malvern Instruments Ltd., Malvern, UK) according to the protocol of Devold et al. (2000). Before size analysis, milk samples were skimmed and diluted in simulated milk ultrafiltrate (Jenness and Koops, 1962) that had been filtered through a 0.2-μm filter (Millipore Corp., Bedford, MA).

Mineral Composition. The content of total Ca, P, Mg, and K was measured by inductive coupled plasma optical emission spectrometry (PerkinElmer optima 5300 DV; Perkin Elmer Inc., Shelton, CT). The samples were decomposed in concentrated, subboiled nitric acid at 250°C in an UltraCLAVE microwave digestion system (UltraCLAVE III; Milestone Inc., Shelton, CT) and diluted 1:10 in concentrated nitric acid before analysis.

Rennet Coagulation Properties. Rennet clotting properties of individual milks were measured by a Formagraph instrument (Lattodinamografo; Foss Italia SpA, Padova, Italy) according to the method described by McMahon and Brown (1982). The milk was pasteurized (at 63°C for 30 min) before cooling to room temperature. The milk (10 mL) was incubated at 32°C for 30 min in the sample cuvette and then 200 μL of rennet (CHY-MAX; Chr. Hansen A/S, Hørsholm, Denmark) diluted 1:50 in acetate buffer (pH 5.6) was added, with immediate start of measurement. The Formagraph was run for 30 min at 32°C and the following parameters were measured: rennet clotting time (**RCT**; min), measured from rennet addition until clotting of the milk started; curd-firming time ($\mathbf{k_{20}}$; min) from the start of clotting until a width of 20 mm between the curves was achieved; and curd firmness ($\mathbf{a_{30}}$), measuring the curd strength after 30 min as the distance (mm) between the curves. All samples were run in triplicate. Some samples did not coagulate and some coagulated but did not obtain a firmness corresponding to 20 mm (k_{20}). In cases where no coagulation (RCT) occurred, a value of 50 min was given, and samples not achieving k_{20} were given a value of 40 min according to Devold et al. (2011).

Statistical Analysis. Statistical analyses on milk-quality parameters were carried out using a mixed-model procedure in SAS (version 9.3; SAS Institute Inc., Cary, NC). The model consisted of the following fixed effects: season (EGS and LGS), forage treatment (PC, PR, HH, and HL), period (the period before onset of feeding experiment and the forage treatment period), genotype (E12-00 and E12-01/11), lactation class (1, 2, 3, 4, and 5), and their interactions. The effect of pen within season and forage treatment and the effect of individual goat within season, forage treatment, and pen were included as random effects. Covariation within animal was accounted for in an analysis of repeated measures. The optimal covariance structure was assessed for each parameter with attention to the Akaike information criterion and Schwarz Bayesian criterion (Littell et al., 1998). Orthogonal contrasts were used to separate the effect of season in the period before onset of forage treatment and to separate the effect of season and forage treatment in the forage treatment period. Pearson correlation coefficients were also calculated between some parameters in milk using SAS.

Principal components analysis (**PCA**) was used to uncover possible relationships among milk components and coagulation properties. Two single partial least squares (**PLS**) regressions were performed using a_{30} and k_{20} as response variables and milk composition as explanatory variables. The categorical factors were set as follows: genotype was set as either 1 or 2 for E12-01/11 and E12-00, respectively; grazing season was set as either 1 or 2 for EGS and LGS, respectively; and the feeding treatment was coded as 1, 2, 3, and 4 for HH, HL, PC, and PR, respectively. The optimum numbers of factors for the PLS regression models were determined through segment-based cross-validation. The Unscrambler V10.1 and V10.2 software (CAMO Software AS, Oslo, Norway) were used for PCA and PLS regression analyses. Data from 3 goats that suffered from mastitis were removed from all data before statistical analysis.

RESULTS

Preforage Treatment Period

Milk composition and rennet coagulation properties of milk from EGS and LGS were analyzed before the goats were assigned to the 4 forage treatments. The results of the preforage treatment period are shown in Table 2.

Milk Composition in the Preforage Treatment Period. The grazing season influenced the milk yield, which was significantly higher (2.81 kg/d) in the EGS compared with the LGS (1.54 kg/d). Total protein and casein content was lower in the EGS compared with the LGS. The mean size of the casein micelles was smaller in LGS (201 nm) than in EGS (219 nm), but no major significant differences in content of individual caseins were observed between the seasons. Calcium and magnesium content were higher in milk from goats in the LGS than the EGS. Milk fat and TS content was higher in LGS (46.4 and 120 g/kg) than in EGS (38.8 and 110 g/kg).

Table 2. Milk composition and rennet coagulation properties of goat milk, showing effects of grazing season [early (EGS) and late (LGS)] and genotype [nonhomozygous (E-12–01/11) and homozygous (E12-00) for the deletion in exon 12 encoding *CSN1S1*] in the preforage treatment period

	Season				Genotype			
Item[1]	EGS (n = 39)	LGS (n = 38)	SEM	*P*-value	E12-01/11 (n = 38)	E12-00 (n = 39)	SEM	*P*-value
Milk yield (kg/d)	2.81	1.54	0.073	***	1.97	2.10	0.063	NS[2]
Total protein (g/kg)	25.5	28.6	0.41	***	29.1	26.7	0.35	***
Casein (g/kg)	17.7	20.6	0.34	***	22.1	19.6	0.30	***
α_{s1}-CN (g/L)	2.69	2.53	0.21	NS	4.44	1.72	0.187	***
α_{s2}-CN (g/L)	3.32	3.43	0.11	NS	3.28	3.35	0.091	NS
β-CN (g/L)	11.9	12.1	0.17	NS	11.8	12.3	0.14	*
κ-CN (g/L)	4.42	4.24	0.16	†	4.43	4.53	0.13	NS
α-LA+ β-LG (g/L)	4.31	4.43	0.121	NS	4.42	4.19	0.091	NS
Casein micelle (nm)	219	201	2.7	***	198	217	2.3	***
Ca (g/kg)	1.07	1.18	0.018	***	1.14	1.09	0.017	†
Mg (g/kg)	0.14	0.15	0.002	**	0.15	0.14	0.002	**
P (g/kg)	1.04	1.01	0.018	NS	1.09	1.02	0.017	**
K (g/kg)	2.02	1.99	0.023	†	2.00	2.00	0.021	NS
Lactose (g/kg)	43.3	43.2	0.38	NS	43.1	43.1	0.32	NS
Milk solids[3] (g/kg)	110	120	1.4	***	116	112	1.1	**
pH	6.67	6.63	0.015	†	6.58	6.61	0.012	NS
SCC ($\log_{10}$/mL)	5.89	6.00	0.015	†	6.00	5.97	0.006	NS
RCT (min)	8.7	10.9	0.93	**	9.5	10.7	0.91	†
k_{20} (min)	23.8	29.2	1.72	*	22.4	30.6	1.45	***
a_{30} (mm)	19.0	16.0	0.82	*	18.6	15.4	0.69	***

[1]RCT = rennet clotting time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min.

[2]NS = nonsignificant at $P > 0.10$.

[3]Milk solids is calculated by adding up fat, protein, and lactose concentration.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Milk from goats homozygous for the deletion in exon 12 (E12-00) had lower protein content (26.7 vs. 29.1 g/kg), casein content (19.6 vs. 22.1 g/kg), TS content (112 vs. 116 g/kg), and larger mean size of the casein micelles (217 nm vs. 198 nm) than heterozygous goats or those without defective alleles (E12-01/11). In addition, E12-00 goats also had a lower content of α_{s1}-CN (1.72 g/L) than milk from E12-01/11 goats (4.44 g/L). The content of α_{s1}-CN in milk from the E12-00 goats ranged from 0.93 to 5.05 g/L, whereas the content ranged from 1.74 to 11.13 g/L in milk from the E12-01/11 goats. The content of caseins other than α_{s1}-CN was slightly higher in milk from E12-00 goats than from E12-01/11 goats.

Rennet Coagulation Properties. Shorter RCT (8.7 vs.10.9 min), shorter k_{20} (23.8 vs. 29.2 min), and higher a_{30} (19.0 vs. 16.0 mm) were obtained in milk from the EGS compared with milk from the LGS. Milk from E12-00 goats had higher RCT and k_{20} (30.6 min) and lower a_{30} (15.4 mm) compared with milk from E12-01/11 goats (k_{20}: 22.4 min; a_{30}: 18.6 mm), meaning that the latter gave a firmer curd faster.

Forage Experimental Period

Milk Yield and Composition. As shown in the preforage treatment period (Table 2), goats in the EGS produced more milk than those in the LGS also during the experimental period (Table 3). The feeding treatments also influenced the milk yield, and the goats on PR had the lowest milk yield in the EGS, whereas goats on HL had the lowest milk yield in the LGS. The contents of total protein (30.7 vs. 26.8 g/kg; $P < 0.001$) and casein (24.*2* vs. 21.0 g/kg; $P < 0.001$) were higher in milk from goats on pasture (PC and PR) compared with milk from goats fed hay (HH and HL), irrespective of grazing season. Milk content of fat (42.6 vs. 34.8 g/kg; $P < 0.001$) and TS (118 vs. 107 g/kg; $P < 0.001$) was also higher on pasture than on hay diets. Goats in HH yielded more milk than goats in HL in both the EGS and LGS. However, no significant differences existed in milk composition and rennet coagulation properties between milk from the goats receiving the 2 hay treatments.

The milk protein and casein content did not vary within the 2 pasture types or the 2 hay qualities. However, the contents and the proportions of individual caseins were influenced by both grazing season and feeding treatments (Table 4; Figure 2). A significantly higher content and proportion of κ-CN and especially α_{s1}-CN characterized the milk from goats on PC in both the EGS and LGS (on average, 3.82 and 5.22 g/L for α_{s1}-CN and κ-CN, respectively) compared with the other treatments. Also, the content and proportion of

Table 3. Milk yield, milk composition, and rennet coagulation properties in goat milk, showing effects of grazing season [S; early (EGS) and late (LGS)], pasture type [P; cultivated pasture (PC) and rangeland pasture (PR)], and hay quality [H; HH = high-quality hay (HH) and low-quality hay (HL)]

		Pasture type		Hay quality			Significance				
Item[1]	Season	PC	PR	HH	HL	SEM	S	PC vs. PR	P vs. H	S vs. P	S vs. P vs. H
n	EGS	10	10	10	9						
	LGS	9	10	9	10						
Milk yield (kg/d)	EGS	2.64	1.95	2.79	2.36	0.146	***	***	NS[2]	NS	†
	LGS	1.65	1.21	1.54	1.03						
Total protein (g/kg)	EGS	30.7	30.0	24.8	26.3	0.81	**	NS	***	NS	NS
	LGS	30.5	31.6	28.8	27.2						
Casein (g/kg)	EGS	24.4	23.8	20.6	20.4	0.68	NS	NS	***	NS	NS
	LGS	23.6	25.0	21.7	21.3						
α-LA + β-LG (g/L)	EGS	4.70	4.06	4.07	3.75	0.235	NS	NS	***	NS	NS
	LGS	4.83	4.70	4.11	3.70						
Casein micelle (nm)	EGS	201	212	221	210	5.3	**	NS	NS	NS	†
	LGS	199	204	196	195						
Ca (g/kg)	EGS	1.17	1.11	1.12	1.17	0.036	**	NS	**	†	**
	LGS	1.09	1.20	1.04	0.97						
Mg (g/kg)	EGS	0.14	0.14	0.15	0.15	0.004	NS	NS	NS	*	NS
	LGS	0.13	0.15	0.15	0.14						
P (g/kg)	EGS	1.08	1.07	1.14	1.13	0.037	NS	NS	NS	NS	NS
	LGS	1.08	1.09	1.06	1.07						
K (g/kg)	EGS	1.93	1.99	2.05	1.91	0.046	NS	NS	NS	NS	NS
	LGS	1.96	2.00	2.05	2.09						
Lactose (g/kg)	EGS	44.1	42.4	42.9	44.1	0.75	NS	NS	NS	NS	NS
	LGS	43.8	42.8	41.5	41.6						
Milk solids[3] (g/kg)	EGS	117	125	104	109	2.7	NS	*	***	NS	NS
	LGS	111	119	106	108						
pH	EGS	6.58	6.54	6.50	6.46	0.03	**	NS	*	NS	NS
	LGS	6.59	6.60	6.57	6.56						
SCC (log_{10}/mL)	EGS	6.10	6.31	5.90	6.06	0.126	NS	NS	NS	NS	NS
	LGS	5.89	5.98	6.07	5.85						
RCT (min)	EGS	11.9	11.5	8.6	8.4	1.85	***	NS	*	NS	†
	LGS	12.0	10.5	11.5	10.1						
k_{20} (min)	EGS	19.1	15.7	18.5	16.1	3.36	***	NS	NS	NS	NS
	LGS	32.8	34.7	36.3	38.7						
a_{30} (mm)	EGS	18.1	17.7	18.1	18.7	1.45	**	NS	NS	NS	NS
	LGS	15.4	16.1	13.3	14.6						

[1]n = number of goats; RCT = rennet clotting time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min.
[2]NS = nonsignificant at $P > 0.10$.
[3]Milk solids is calculated by adding up fat, protein, and lactose concentration.
†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

α_{s2}-CN in milk was influenced by both treatment and season, with higher content in the EGS (3.68 g/L) than in the LGS (2.89 g/L), and higher in milk from goats on pasture (3.48 g/L) than goats fed hay (3.04 g/L). The content of β-CN was higher in the EGS (12.51 g/L) than the LGS (11.54 g/L) and highest in milk from goats on PR (13.07 g/L and 54.2% of total casein, respectively) and, on average, higher on pasture (12.39 g/L) than on hay (11.66 g/L; Table 4; Figure 2). The pasture effect was stronger in the EGS than in the LGS, as indicated by the significant season versus pasture versus hay contrast. Milk fat (47.7 vs. 37.6 g/kg; $P < 0.01$) and TS (122 vs. 114 g/kg; $P < 0.05$) were higher in goats on PR than those grazing PC. The mean size of the casein micelles was larger in the EGS (211 nm) compared with the LGS (199 nm), and negatively correlated with the content of total protein, casein, and α_{s1}-CN (Table 5). The content of milk minerals, except for calcium, was not significantly influenced by the grazing season or feeding treatments. The content of calcium was higher in milk from goats in the EGS compared with the LGS and higher when the goats were fed pasture than hay (Table 3) and positively correlated with the content of α_{s2}-CN, β-CN, and lactose (Table 5). The pH of the milk was slightly higher in the LGS (Table 3).

Rennet Coagulation Properties. Season significantly affected milk coagulation properties (**MCP**), whereas the effects of feeding treatments were only minor. Season had a large effect on k_{20}, which was almost twice as long in the LGS compared with the EGS (Table 3). In the LGS, very few milk samples achieved the value of k_{20}. None of the milk samples from the E12-00 goats obtained k_{20} in the LGS (results not shown). Curd firmness was also affected by graz-

Table 4. Effect of grazing season [S; early (EGS) and late (LGS)], pasture type [P; cultivated pasture (PC) and rangeland pasture (PR)], and hay quality [H; high-quality hay (HH) and low-quality hay (HL)] on contents of individual caseins (g/L) in goat milk

		Pasture type		Hay quality			Significance				
Item	Season	PC	PR	HH	HL	SEM	S	PC vs. PR	P vs. H	S vs. P	S vs. P vs. H
n[1]	EGS	10	10	10	9						
	LGS	9	10	9	10						
α_{s1}-CN	EGS	3.72	2.29	2.60	2.36	0.413	NS[2]	**	NS	NS	NS
	LGS	3.92	2.84	3.04	3.04						
α_{s2}-CN	EGS	3.69	3.95	3.42	3.44	0.213	***	NS	**	NS	NS
	LGS	3.08	3.20	2.86	2.42						
β-CN	EGS	12.5	13.8	12.1	11.6	0.33	***	***	**	NS	*
	LGS	10.9	12.3	11.5	11.4						
κ-CN	EGS	5.56	4.89	4.46	4.39	0.303	NS	*	†	NS	NS
	LGS	4.88	4.13	4.68	4.28						

[1]n = number of goats.

[2]NS = nonsignificant at $P > 0.10$.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ing season: milk from all treatments had reduced a_{30} in the LGS compared with the EGS (Table 3). Milk from goats fed HH had the largest reduction (from 18.1 to 13.3 mm), whereas milk from goats feed PR had the lowest reduction (from 17.7 to 16.1 mm). However, these differences were not significant. Milk from goats on PC and PR had longer RCT compared with milk from goats fed HH and HL in the EGS, and milk from goats on PC also had the longest RCT in the LGS (Table 3). No significant differences between the feeding treatments on k_{20} and a_{30} were observed. A strong negative correlation between a_{30} and both RCT and k_{20} was found. Curd firmness was also positively correlated with protein yield and contents of caseins, especially α_{s1}-CN, α_{s2}-CN, and β-CN, in addition to lactose and calcium content (Table 5).

Multivariate Analysis of Milk Composition and Rennet Coagulation Properties. The PCA of the milk composition and coagulation properties of the samples is presented in Figures 3 and 4. Principal component (**PC**) 1 and 2 explained 50% of the variation (Figure 3) and, together with PC 3 (Figure 4), 62% of the total variation was explained. The relationship between the variables is shown in the correlation loading plot (Figures 3A and 4A), whereas the corresponding score plots (Figures 3B and 4B) show the distribution of the milk samples.

Milk samples of goats in the EGS [Figure 3B, gray (early)] were characterized by higher milk yield, a higher content of α_{s2}-CN, calcium, and lactose, in addition to better rennet coagulation properties as higher a_{30} and shorter k_{20} compared with milk samples collected in the LGS (black; Figure 3B). The score plot of PC 1 and PC 3 (Figure 4B) separated the milk properties from the hay and pasture treatments in 2 groups. Milk from goats on pasture (PC and PR; gray) was characterized by a higher content of α_{s2}-CN, β-CN, and calcium content compared with milk samples from goats fed hay (HH and HL; black).

Partial least squares regression was used to investigate the influence of milk composition on MCP (a_{30} and k_{20}). The PLS regression coefficients for a_{30} and k_{20} are presented in Figures 5 and 6, respectively. The content of lactose, calcium, α_{s2}-CN, β-CN, and casein in the goat milk had a significant positive influence on a_{30}, whereas LGS and genotype (deletion in exon 12) had a significant negative influence on a_{30}. The contents of α_{s2}-, β-, and κ-CN in the milk were significantly negatively correlated with k_{20}, whereas season (LGS) and genotype (E12-00) were positively correlated with k_{20}. As a short time until the achieved k_{20} is reached is desirable, high contents of α_{s2}-, β-, and κ-CN are regarded as positive attributes, whereas the LGS and E12-00 then where regarded as negative.

DISCUSSION

The present study focused on the main hypothesis that ad libitum access to high-quality cultivated pasture would yield higher milk protein and casein content and, thus, improve the MCP compared with milk produced by goats grazing free range in forest pasture. Furthermore, we also expected that the grazing season would not influence MCP and the content of casein and protein in milk from goats grazing PC, but would decline in milk from goats grazing on PR due to decreased forage availability and quality in the latter. A third hypothesis was that the individual casein composition would not be affected by the forage treatment or the grazing season. Two hay qualities were included as control feeds, as hay quality does not change during the course of time as pasture does. As most dairy

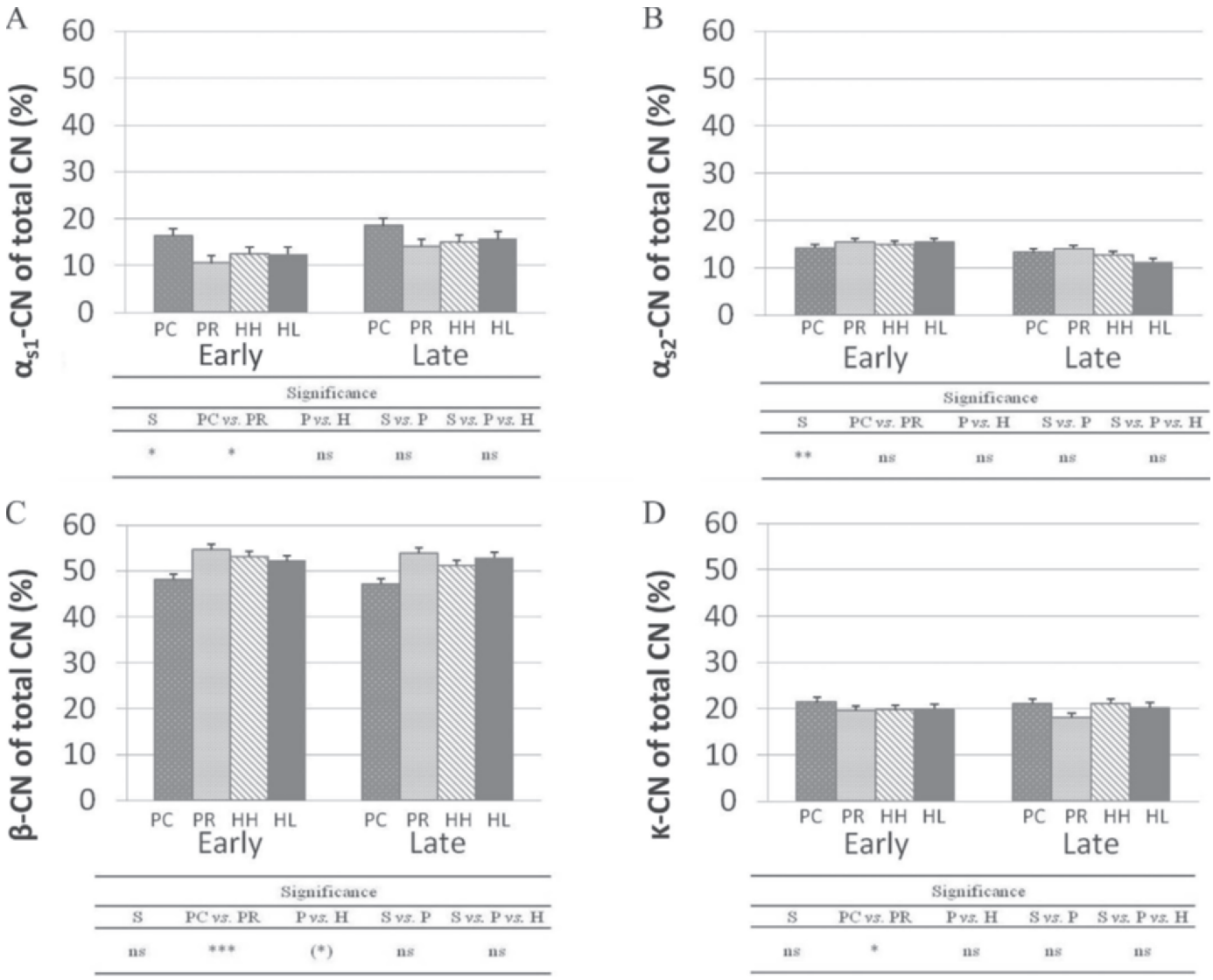

Figure 2. Casein composition of individual caseins (LSM) in goat milk given in percentage of total casein. Effect of grazing season (S; early and late), forage [pasture (P); PC = cultivated pasture; PR = rangeland pasture], and hay (H; HH = high-quality hay; HL = low-quality hay). (A) Proportion of α_{s1}-CN; (B) proportion of α_{s2}-CN; (C) proportion of β-CN; (D) proportion of κ-CN. Error bars represent the SEM. ns = nonsignificant at $P > 0.10$; (*) = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

goat farmers in Norway depend on pastures as a feed resource and as mating and kidding season are easily manipulated, our findings have considerable applied relevance.

Our results revealed a higher milk yield in the EGS than in the LGS. Milk yield was 38% reduced in the LGS compared with the EGS in the 2 grazing treatments. In the HH and HL treatments, the reduction in milk yield from the EGS to LGS was 45 and 56%, respectively. This may be partially explained by the preforage treatment feeding regimen and by the season itself. The seasonal effect was confounded by the preforage treatment feeding regimen, as the goats in the EGS went directly from indoor silage to the forage experimental period, whereas the goats in the LGS were out on rangeland pasture for about 50 d before they were allocated to their respective forage treatments. Ideally, the LGS goats should have been kept indoors on silage from the start of lactation until the start of the forage treatment experiment. This was not possible for practical reasons, such as silage availability. However, the goats in the EGS and LGS were in same stage of lactation.

Higher contents of protein and casein in milk from goats on pasture than from those on hay diets were probably due to a higher dietary concentrate-to-forage

Table 5. Pearson correlation coefficients of some goat milk parameters[1]

Parameter[2]	Lactose	RCT	k_{20}	a_{30}	CN	α_{s1}-CN	α_{s2}-CN	β-CN	κ-CN	Micelle	Ca
Protein	−0.00	0.29***	−0.18*	0.07	0.83***	0.61***	0.06	0.10	0.39***	−0.49***	0.25**
Lactose		−0.34***	−0.28***	0.36***	0.11	0.14	0.36***	0.29***	−0.03	−0.14	0.39***
RCT			0.33**	−0.64***	0.14	0.01	−0.13	−0.17*	0.21*	0.01	−0.18*
k_{20}				−0.79***	−0.19*	−0.28***	−0.35***	−0.26***	−0.22**	0.14	−0.19*
a_{30}					0.08	0.27***	0.27***	0.23**	0.05	−0.14	0.20*
CN						0.59***	0.04	0.16	0.20*	−0.56***	0.28***
α_{s1}-CN							−0.08	−0.13	0.18*	−0.63***	0.16
α_{s2}-CN								0.57***	0.17*	0.11	0.42***
β-CN									0.18*	0.04	0.28***
κ-CN										−0.09	−0.01
Micelle											−0.22**

[1]Data from both preforage treatment period and forage experimental period are included (n = 154).

[2]RCT = rennet clotting time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min; micelle = mean size of casein micelles.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ratio on pasture than on hay (Steinshamn et al., 2014), as increasing energy intake has been shown to have a positive effect on milk protein content (Morand-Fehr and Sauvant, 1980). Likewise, the goats grazing PC spent less energy on locomotion and had more energy available for milk production than the goats grazing on PR. The goats on PR walked, on average, 5.0 and 7.5 km daily in the EGS and LGS, respectively, whereas the PC was smaller in area and located close to the barn (Steinshamn et al., 2014). Estimated energy balance indicated that goats on PR had lower energy balance (−1.6 MJ of NE_L/d, on average) than goats on PC (4.3 MJ of NE_L/d, on average; Steinshamn et al., 2014).

It is important to establish the factors influencing MCP when the milk is supposed to be used in cheesemaking. In cow milk, the casein composition is known to influence the MCP (St-Gelais and Haché, 2005; Wedholm et al., 2006; Jõudu et al., 2008), but the factors influencing casein composition, except genotype, is less known in both goat and cow milk. In the present study, the composition of individual caseins was influenced both by the feeding treatments and genotype. Particularly interesting was the higher content and proportion of α_{s1}- and κ-CN in milk from PC compared with the other treatments. A high content and proportion (in total casein) of κ-CN is desired in cow milk used for cheese production (Wedholm et al., 2006; Jõudu et al., 2008), whereas goats with low synthesis of α_{s1}-CN is known to produce milk with reduced MCP (Ambrosoli et al., 1988; Zullo et al., 2005; Devold et al., 2011). Milk from goats in the EGS grazing PR had a high content of κ-CN, but apart from longer RCT, a clear positive effect of an increased content of κ-CN on the MCP was difficult to observe. Milk from goats on PR had the highest content and proportion of β-CN, irrespective of season. Enrichment with β-CN powder reduced MCP in cow milk (St-Gelais and Haché, 2005). In goat milk, an increased proportion of β-CN could be associated with reduced MCP, because a reduction in α_{s1}-CN was partly compensated by the other caseins and especially β-CN. Thus, the reduced MCP is more likely to be caused by a reduced content of α_{s1}-CN rather than by the increased content of β-CN (or by a combined effect). A high proportion of α_{s2}-CN has been reported to be associated with noncoagulating cow milk in an Estonian study (Jõudu et al., 2008); however, our results showed a positive influence of α_{s2}-CN on k_{20} and a_{30}. The correlation between the content of α_{s2}-CN and the curd-firming properties of goat milk during coagulation has not, to our knowledge, been reported previously. Milk from goats grazing pasture (PC and PR) had a higher content of α_{s2}-CN than those fed hay (HH and HL). In the LGS, milk from all treatments had lower relative amounts of α_{s2}-CN and higher relative amounts of α_{s1}-CN compared with the EGS season. The effect of diet on the casein content has been referred to in other studies [e.g., Bonanno et al. (2013) and Valenti et al. (2012)] that reported higher milk yield and casein content in milk from goats fed a high-energy diet. Valenti et al. (2012) studied the interaction between genotype and diet and reported a higher daily production [milk yield × α_{s1}-CN (g/kg of milk)] of α_{s1}-CN when the goats homozygous for the strong allele (AA) were fed a high-energy diet than if goats homozygous for the weak allele (FF) were fed the same diet. However, the concentration of α_{s1}-CN in milk (g/kg) and the relative amount of the individual caseins (in total casein) were not significantly different, in contrast to the present study. Apart from the study of Valenti et al. (2012), a dietary effect on the caprine casein composition has not, to our knowledge, been reported previously. A few studies have been conducted on this subject with dairy cows, where it has been shown that the κ-CN proportion in the total casein is adversely affected by poor energy supply (Christian et al., 1999; Coulon et al., 2001; Leiber et al., 2005) and higher content of κ- and

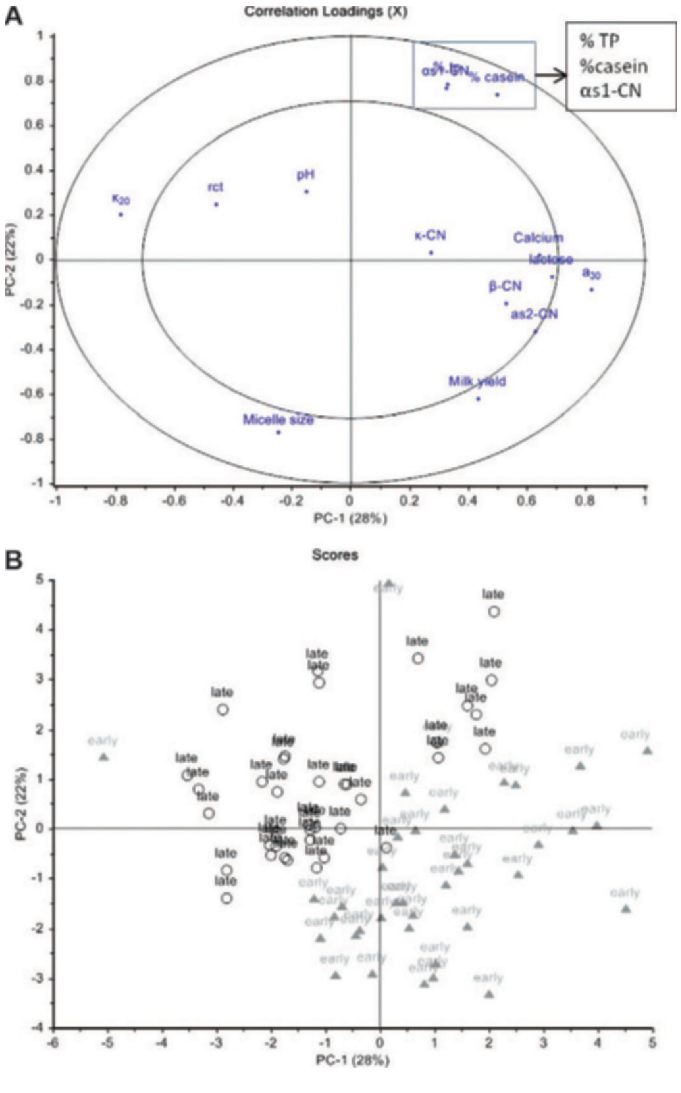

Figure 3. Principal components (PC) analysis of milk composition and rennet coagulation properties in relation to grazing season (PC-1 and PC-2). (A) Correlation loading plot of variables; (B) score plot. Samples are grouped according to season: 1 (gray) = early; 2 (black) = late. TP = total protein; rct = rennet clotting time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min. Color version available in the online PDF.

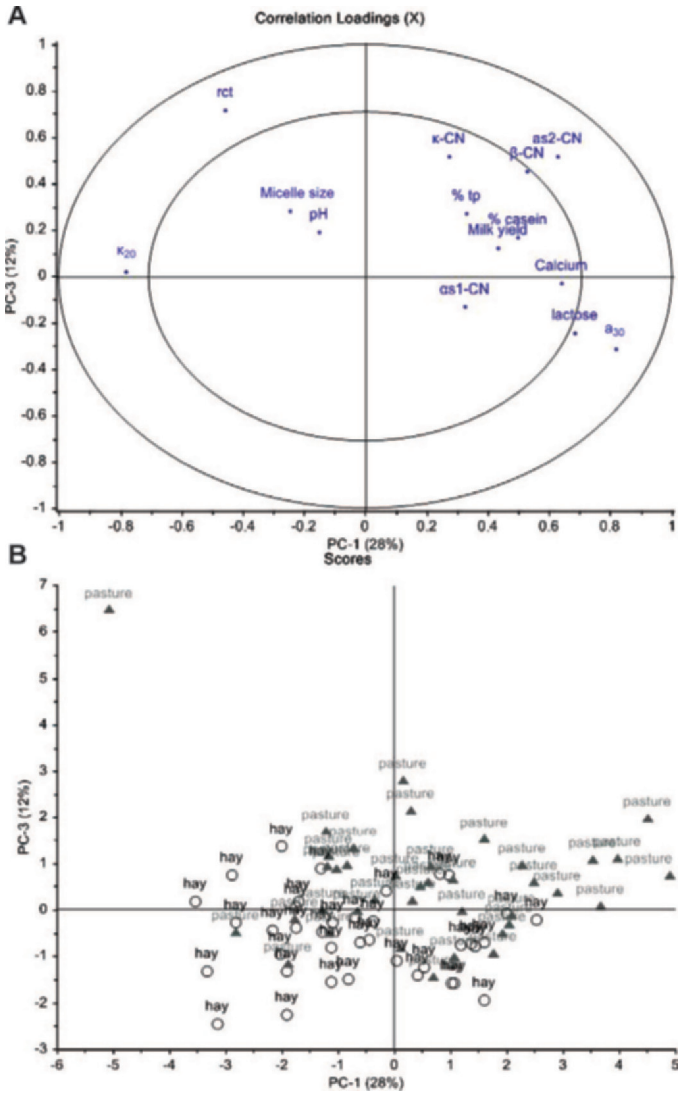

Figure 4. Principal components (PC) analysis of milk composition and rennet coagulation properties in relation to forage type (PC-1 and PC-3). (A) Correlation loading plot of variables; (B) score plot. Samples are grouped according to forage. Black = hay [high-quality hay (HH) and low-quality hay (HL)]; gray = pasture [cultivated pasture (PC) and rangeland pasture (PR)]. tp = total protein; rct = rennet clotting time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min. Color version available in the online PDF.

β-CN has been found in milk produced indoors than on pasture (Stergiadis et al., 2012). In contrast to the results obtained in our study, a lower content of α_{s1}-CN was observed in milk from cows fed a high-energy pasture diet (Christian et al., 1999). Jõudu et al. (2009) reported that cow milk obtained a stronger curd when the proportion of α_{s2}- and β-CN in total casein was lower or the ratio of κ-CN to other caseins was higher. A high content of α_{s2}-CN and low content of κ-CN were associated with poor- or noncoagulating milk, which is in accordance with the results of Wedholm et al. (2006), who also found a correlation between content and proportion of κ-CN and poor or noncoagulating milk. Unfortunately, the latter study did not report the content of α_{s2}-CN.

Despite a higher protein and casein content (except PC), milk from goats in the LGS displayed poorer rennet coagulation properties by showing a longer k_{20} and a lower a_{30}. Glantz et al. (2010) reported higher a_{30} when the casein micelles were small in size (cow milk), but this relationship between a_{30} and micelle size could not be established in our study. In general, the mean casein micelle size is negatively correlated with

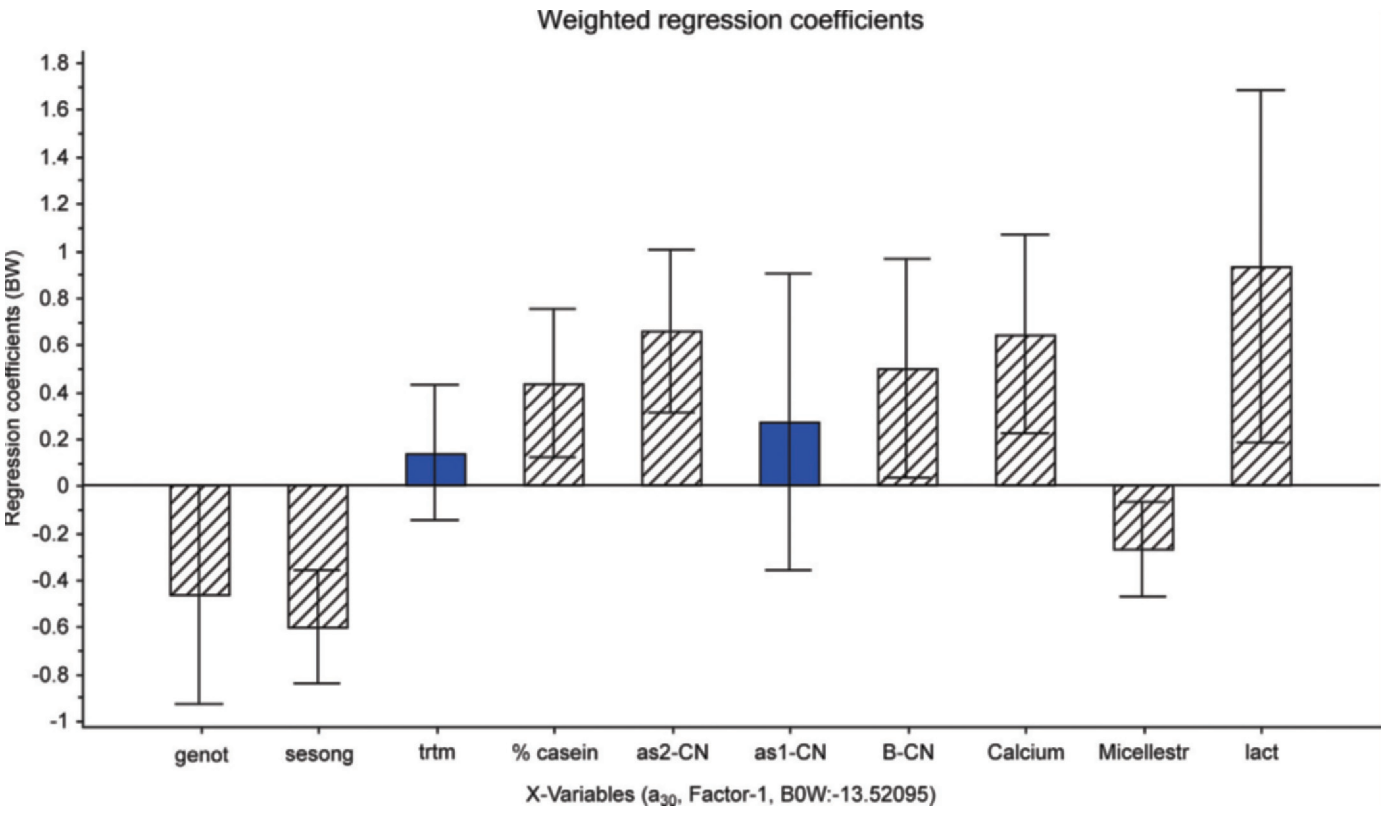

Figure 5. Partial least squares regression coefficients for some selected factors and their influence on curd firmness (a_{30}) in goat milk. Striped bars show significant factors for a_{30}. The error bars represent the uncertainty limits of the regression coefficients. genot = genotype; sesong = season; trtm = treatment; Micellestr = micelle size; lact = lactose; B0W = regression coefficient of the intercept. Color version available in the online PDF.

the content of κ-CN (Dalgleish et al., 1989); however, in the current study, the size of the casein micelles were not negatively correlated with the content of κ-CN but with the content of α_{s1}-CN. This result is in accordance with earlier studies on goat milk (Pierre et al., 1998; Devold et al., 2011), and may explain the smaller mean size of the casein micelles in the LGS, as the content and proportion of α_{s1}-CN were higher for all treatments in this season. As expected, goats homozygous for the deletion in exon 12 (E12-00) had poorer MCP compared with goats having 1 or 2 nondefective alleles (E12-01/11). The longer k_{20} and lower a_{30} are in accordance with previous Norwegian studies (Devold et al., 2011) and has also been reported for weak alleles in other breeds (e.g., Cilentana; Zullo et al., 2005) and Saanen and Alpine (Ambrosoli et al., 1988). However, we did not expect the content of α_{s1}-CN in milk from of the E12-00 goats to vary in the range of 0.93 to 5.05 g/L. In fact, these goats seem not to have been "true null," as indicated in previous studies (Vegarud et al., 1999; Devold et al., 2011), even though the content of α_{s1}-CN was extremely low compared with strong genotypes such as, for example, Ethiopian goats (Mestawet et al., 2013) and Girgentana goats (Valenti et al., 2012). Because the frequency of the defective allele encoding α_{s1}-CN has been very high (0.73) in the Norwegian goat population (Hayes et al., 2006), different casein compositions in milk related to feeding pasture and hay in the present study were very interesting. Goats grazing PC increased their content of α_{s1}-CN in milk, independently of genotype; however, the effect of increased α_{s1}-CN in the milk did not affect the MCP notably. This observation could imply that also other factors are important for MCP. The protein that may be the most important regarding MCP seems to be α_{s2}-CN, as the content of this protein was lower in milk from all feeding groups in the LGS, in a similar pattern as a_{30}. Milk from goats grazing PR had the smallest reduction in a_{30} from the EGS to LGS, and this milk also had the highest content of α_{s2}-CN. A reduction in the calcium content was observed in milk from all treatments except PR in the LGS. Calcium is, in addition to hydrophobic interactions, important for the interactions between and within the casein molecules and the casein micelles and is, therefore, correlated with a_{30}. The content of lactose was also correlated with a_{30}, as

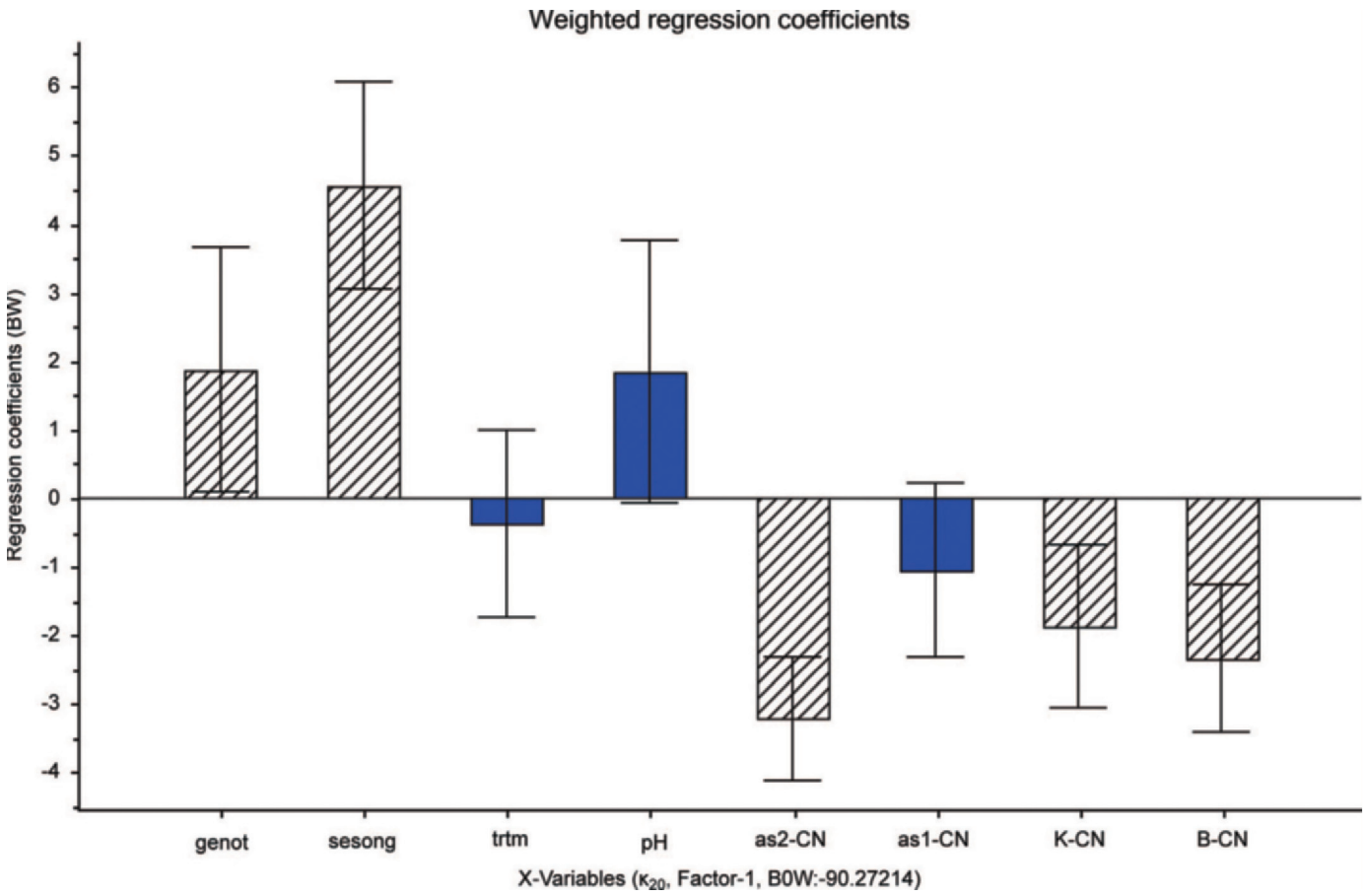

Figure 6. Partial least squares regression coefficients for some selected factors and their influence on firming time (k_{20}) in goat milk. Striped bars show significant factors for k_{20}. The error bars represent the uncertainty limits of the regression coefficients. genot = genotype; sesong = season; trtm = treatment; B0W = regression coefficient of the intercept. Color version available in the online PDF.

also shown by others (Superchi et al., 2005; Todaro et al., 2005; Leitner et al., 2011; Pazzola et al., 2012); however, it is not likely that the lactose content itself improves a_{30}. The connection between the content of lactose and the a_{30} and k_{20} is probably an indicator of an unexplained correlation.

The variation in a_{30} between the individual goat milk samples ranged from 0 to 30 mm, and even for the E12-01/00 goats, the majority of samples had a lower a_{30} compared with what has been reported in the literature. An average a_{30} of 25 mm has been reported for milk from Girgentana goats (Todaro et al., 2005). The highest a_{30} ever reported for goat milk was probably shown by Mestawet et al. (2013), who observed an a_{30} of 45 mm in milk from a local Ethiopian breed. The reason for the reduced curd-firming properties in the LGS warrants further investigation, as the differences in milk components measured in the current study hardly can explain the impaired curd-firming properties completely. Degradation of caseins by plasmin may infer the a_{30} and reduce the cheese yield (Bastian and Brown, 1996). In addition to impaired MCP, the milk yield was reduced in the LGS. Plasmin may cleave off a peptide from β-CN (fragment 1–28) that blocks the K^+ channels of the mammary epithelial cells, which is associated with reduced milk yield (Silanikove et al., 2000). A reduction in the content of α_{s2}- and β-CN in the LGS may indicate degradation of these caseins by plasmin, which is known to impair the clotting properties of milk (Fantuz et al., 2001; Leitner et al., 2006). Factors such as stress and late lactation are known to increase the plasmin activity in milk (Silanikove et al., 2000; Fantuz et al., 2001); however, the goats in our experiment were in the same lactation stage in both the EGS and LGS.

Further selection of goats without the deletion in exon 12 (*CSN1S1*) and selection of goats producing milk with good MCP is important for increasing the milk quality for cheese production. Moreover, pasture increases the content of protein, casein, α_{s1}-CN, α_{s2}-CN, and calcium, which influences MCP positively.

CONCLUSIONS

The present study showed that milk composition and casein composition were influenced by dietary treatment. Pasture (cultivated and rangeland) increased the casein and protein content in the milk, which is favorable with respect to cheese yield. The casein composition in milk from goats grazing PC had a high content of α_{s1}- and κ-CN, known to increase the rennet coagulation properties in cow milk. However, the rennet coagulation properties of the milk were not prominently influenced by the feeding treatment, whereas these properties were negatively affected by LGS. Also, milk yield was reduced in the LGS, whereas the content of casein was unchanged. The reason for the reduced milk yield and rennet coagulation properties in the LGS should be further investigated.

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Effect of forage type and season on Norwegian dairy goat milk production and quality☆

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ABSTRACT

Goat milk quality produced by goats grazing on rangeland may have inferior quality with low content of solids and high content of free fatty acids (FFA). The main objective of this experiment was to test the effect of grazing woodland or cultivated pasture on dairy goat milk production and quality in early (EGS) and late (LGS) grazing seasons. Two different hay qualities (high and low quality) were used as control feeds. Eighty Norwegian dairy goats were grouped according to genotype and lactation number and randomly divided into two groups with approximately 8 weeks difference in kidding date. The EGS and LGS feeding experiments had 8 weeks departure in time, when the goats in the two kidding groups were in the same stage of lactation, on average 132 (SD 11.5) days in milk. The goats in each group were randomly allocated to four forage treatment groups: WR, woodland rangeland; PC, cultivated pasture; HH, high quality hay; HL, low quality hay. Goats on WR yielded less milk (1.58 *vs.* 2.15 kg/d, $P < 0.001$) but with higher milk fat (47.7 *vs.* 37.6 g/kg, $P < 0.01$) and total solids content (122 *vs.* 114 g/kg, $P < 0.05$) than goats on PC. Milk FFA content was not affected ($P > 0.1$) by pasture type. The effects of pasture type on milk yield and milk gross composition were similar in EGS and LGS, but milk yield (2.44 *vs.* 1.36 kg/d, $P < 0.001$) and milk content of FFA (0.35 *vs.* 0.23 mEq/L, $P < 0.05$) were higher in EGS than LGS. Grazing resulted in similar milk yield but higher milk fat (42.6 *vs.* 34.8 g/kg, $P < 0.001$), protein (32.3 *vs.* 29.6 g/kg, $P < 0.001$) and total solids (118 *vs.* 107 g/kg, $P < 0.001$) content and tended to yield lower content of FFA (0.23 *vs.* 0.34 mEq/L, $P = 0.068$) than hay diet. The milk from the goats on WR had lower ($P < 0.05$) proportion of medium-chain fatty acids (FA), C10:0–C14:0 and C18:2c9t11, but higher ($P < 0.05$) proportion of C18:0, C18:2c9,12 and C20:0 than on PC. Grazing compared to hay feeding resulted in milk with lower proportion of medium-chained FAs (C12:0–C14:0) and C16:0 and higher proportion of the long-chained FAs C18:0, C18:1t11, C18:2c9,t11, C18:3c9,12,15, C20:0 than hay feeding. The milk proportion of the short- and medium-chained FAs (C6:0–C14:0) and C16:0 was higher ($P < 0.0001$) in LGS than in EGS, whilst the proportion of long chained FAs (C18:0, C18:1c9, C18:1t11, C18:2c9,12, C18:2c9t11 and C18:3c9,12,15) were lower ($P < 0.001$). In conclusion, woodland rangeland yielded less milk than cultivated pasture but milk gross composition and content of FFA were not altered.

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1. Introduction

Dairy goat milk quality in Norway is variable, and it often does not meet the requirements of the industry for cheese making. Lipolysis and high content of free fatty acids (FFA) and tart and rancid off-flavour are among the main problems. Much of the inferior milk quality may be ascribed to genetic factors. The Norwegian dairy goat breed has a very high frequency (>0.70) of a defective allele with a single nucleotide deletion in exon 12 of the gene encoding α_{s1}-casein in milk (Hayes et al., 2006; Dagnachew et al., 2011). Milk from goats with deletion in exon 12 α_{s1}-casein gene has reduced protein and fat content (Dagnachew et al., 2011), low or no α_{s1}-casein and poor rennetability (Devold et al., 2010). Milk from goats that have "weak" variants of α_{s1}-casein is also more prone to lipolysis and high content of FFA (Chilliard et al., 2003; Dagnachew et al., 2011). As long as goat milk was used for the traditional whey product 'brown cheese', inferior clotting properties and high FFA content were not major problems. However, the interest for rennet- and acid coagulated cheeses by Norwegian industries and consumers is increasing, and production of these types of cheeses requires milk of a different quality.

Traditionally, goat milk production in Norway is seasonal with kidding in winter and early spring and with peak milk production during the summer grazing season. During the grazing season, goats to a large extent graze natural unimproved grasslands or graze free range in woodlands and mountains. The forage quality of rangeland herbage is variable and declines during the grazing season due to the phenological development of the grazed plants (Lunnan and Todnem, 2011). Reduced allowance and decreased quality of herbage, together with underfeeding and consequently negative energy balance, are also assumed to contribute substantially to the problems of high FFA content and off-flavour (Eknæs and Skeie, 2006). Supporting this, the milk content of FFA (recorded by the Norwegian Goat Milk Recording System, Blichfeldt personal communications) is highest during the summer months. Increased FFA in milk during the grazing season has also been observed in experimental trials (Eknæs et al., 2006; Eknæs and Skeie, 2006). Off-flavour occurs in periods when dry matter (DM) content in milk is low, and a number of studies have revealed a negative correlation between off-flavour and milk DM content (Rønningen, 1965; Bakke et al., 1977; Skjevdal, 1979). It is therefore recommended to supplement goats on pasture with concentrate, preserved forages, vegetable oils and fat-enriched concentrates. Particularly oil and fat supplementation has shown to be efficient in decreasing the frequency of off-taste, lipolysis and the concentration of milk FFA (Skjevdal, 1979; Astrup et al., 1985; Chilliard et al., 2003; Eknæs et al., 2009). Chilliard et al. (2003) found that the lipoprotein lipase (LPL) activity decreased with increasing milk C16:0 proportion ($r = -0.70$). Diets that increase the milk fat proportion of C16:0 and reduce the proportion of C6:0–C10:0 reduce the frequency of rancid and tart flavours (Eknæs et al., 2009) and the level of FFA (Astrup et al., 1985). However, it has also been found that high C16:0 and low C18:1c9 proportion in milk and a high energy balance may be related to high FFA content and off-flavours (Dønnem et al., 2011b). It is well known that grazing has strong impact on milk fatty acid (FA) composition by decreasing saturated FA and increasing FA considered beneficial, like C18:1c9, C18:3c9,12,15 and C18:2c9t11, in goats when compared to diets based on preserved forages and concentrates (Tsiplakou et al., 2006; Chilliard et al., 2007; Renna et al., 2012b). However, less is known on how rangeland, as used in Norway, influence milk FA profile and milk content of FFA. Eknæs and Skeie (2006) found that goat milk sensory quality improved (less rancid taste) and milk content of FFA decreased when hay, fed *ad libitum*, replaced rangeland for a short period (2 d). They also found that when the goats later grazed a cultivated pasture after rangeland, the milk quality improved with reduced FFA content. It is known that the lipoprotein lipase activity and lipolysis are more pronounced during mid-lactation (3–6 months) than at early (<2 months) and late lactation (Chilliard et al., 2003). Mid-lactation coincides with the time goats traditionally are on pastures in Norway. Therefore, the effect of forage type (rangeland) or grazing season on milk quality is confounded with the effect of lactation stage.

The aim of this study was to unravel some of these discrepancies, by testing the effects of forage type and quality, *i.e.* rangeland *vs.* cultivated pasture or grazing *vs.* hay, on goat milk production and milk quality. Additionally, we tested whether there was a seasonal effect (early and late season) of forage quality on the same production traits with goats in the same stage of lactation. We used two hay types, with known quality (high and low), as controls as pasture quality changes during the grazing season.

2. Materials and methods

2.1. Animals, experimental design and management

Eighty Norwegian dairy goats at Senja videregående skole, Norway (N 69°21.397′, E 17°56.319′) were blocked according to genotype (with and without double deletion in exon 12 of the gene encoding α_{s1}-casein (CSN1S1)) and lactation number (5 groups; 1–4 according to lactation number, group 5 = more than 4 lactations) before mating and randomly divided into two groups: early grazing season (EGS) and late grazing season (LGS) with approximately 8 weeks difference in mating time. The genotyping was performed according to Hayes et al. (2006). Average kidding date was February 2 (SD 9 d) and April 1 (SD 12 d), 2010. At the start of the grazing season, June 28th, the goats within each of the two groups, EGS and LGS, blocked for genotype (with and without deletion in exon 12 of the CSN1S1 casein gene) and lactation number (1–5), were randomly allocated to four forage treatment groups: PC = cultivated pasture, WR = woodland rangeland, HH = High quality hay, HL = low quality hay. Thus, the design applied was a 2 × 4 factorial with season (EGS and LGS) as one factor and forage type (PC, WR, HH and HL) as the other factor, with 10 goats in each treatment. The 10 goats in each forage treatment were randomly divided into two sub-groups (pens) with 5 goats, accounting for genotype and lactation number. In the two hay treatments the five goats in each group within treatment were kept indoors in separate pens (*i.e.* two replicates per treatment), while the sub-groups of goats within each pasture treatment grazed together.

The EGS goats went directly from the indoor silage based ration to their respective forage treatment groups on the 28th of June 2010. The 40 goats in the LGS group grazed together with the WR group of the EGS goats until the 16th of August 2010 before they were allocated to their respective forage treatment groups. The forage treatment periods lasted for 3 weeks.

All goats were machine milked twice a day at 06:30 and 16:00 h. Concentrate was distributed in equal amounts two times per day at each milking. The goats were weighed for three consecutive days in the week before they entered the feeding treatments (June 21–23 and August 9–11

in EGS and LGS, respectively) and in the week after commencement of the forage treatment periods (July 21–23 and September 8–10 in EGS and LGS, respectively) when they were on the same diet.

Data from three goats that suffered from mastitis were removed from all data. The milk sample for fatty acid analysis for one goat on treatment PC in EGS could not be analysed.

The procedures in the experiment were according to the regulations set by the Norwegian Animal Research Authority, Oslo, Norway (http://www.mattilsynet.no/fdu/), which is in accordance with EU Directive (86/609/EEC) and the European convention (ETS No. 170) on the protection of animals used for experimental and other scientific purposes.

2.2. Feeding and grassland characteristics

From kidding until start of the grazing season (28th of June 2010) the goats received the same diet: silage fed *ad libitum* [net energy for lactation (NEL) = 5.91 (SD 0.42) MJ/kg DM; crude protein (CP) = 138 (SD 18) g/kg DM; neutral detergent fibre (NDF) = 539 (SD 49) g/kg DM] and 1.1 kg concentrate (as fed) per day per head. The concentrate contained the following ingredients (g/kg): barley (278), oat (263), wheat bran (159), sugar cane molasses (65), sugar beet pulp (60), extracted soybean meal (46), oil seed (41), Soy Pass® (34), limestone (19), and other minerals and vitamins (35). During the forage treatment periods, all goats were supplemented with the same concentrate mixture as fed indoors, applied at a rate of 0.9 kg DM per day per head.

The cultivated pasture (PC) was a ley (2.1 ha in area and 10 m above sea level) in its first production year dominated by the sown grasses (proportion of DM yield in EGS/LGS) *Phleum pratense* L. (0.60/0.45), *Festuca pratensis* L. (0.05/0.09) and *Poa pratensis* L. (0.04/0.06), with some unsown species such as *Elytrigia repens* L. (0.03/0.22), *Stellaria media* L. (0.16/0.12) and *Matricaria matricarioides* Porter ex Britton (0.07/0.02) as assessed by the dry weight rank method (Mannetje and Haydock, 1963). The estimated daily allowance was on average 13 and 30 kg DM/goat in EGS and LGS, respectively. The high allowance was given to ensure that the goats could select high quality forage. After finishing the grazing in EGS the field was cut and the regrowth was used in the in LGS experiment. The dominating grass species, *P. pratensis*, was in the booting stage at the start of EGS and in vegetative regrowth stage in LGS. The rangeland (WR) was approximately 450 ha, ranging from 65 to 265 m above sea level, with the following vegetation types (% of land area): blueberry-birch woodland (41), fen (22), meadow-birch woodland (21), lichen/heather-birch woodland (6), wet woodland (4), natural grassland (3) and spruce woodland (2). Dominating species were birch (*Betula pubescens* Ehrh.), bilberries (*Vaccinium myrtillus* L.), Swedish cornel (*Cornus suecica* L.), wavy hair-grass (*Avenella flexuosa* L.) and sweet vernal grass (*Anthoxanthum odoratum* L.). The hay was produced from the first cut of a ley in 2009. The dominating species were *P. pratense* (0.61) and *F. pratensis* (0.27). The HH quality was cut at early booting stage and HL at beginning of flowering of *P. pratense*. The hay was fed *ad libitum* twice daily after milking allowing a residue of 10%. The goats on pastures grazed day and night.

2.3. Data collection and sampling

2.3.1. Milk yield, sampling and analysis

Individual milk yield was measured and individual milk samples were collected twice in each grazing periods; (1) the week (6–8 d) before goats were allocated to the forage dietary treatments [on average 137 (SA 9) and 127 (SA 12) days in milk (DIM) in EGS and LGS, respectively], and (2) on days 14–16 after onset of the dietary treatment [on average 158 (SA 9) and 148 (SA 12) DIM in EGS and LGS, respectively]. The milk samples were collected during morning and evening for three consecutive days. The samples were stored at 4 °C and four samples from the first two days in each period were pooled into one sample for each goat and period. The pooled samples were split in two: one added a tablet of Bronopol (2-bromo-2-nitropropane-1,3-diol; D&F Inc., USA) and was analysed for fat, protein, lactose, urea and FFA content with an infrared milk analyser (Milkoscan 6000, Foss-Electric, Hillerød, Denmark) and the other parallel was used for extraction of fat and further analysis of fatty acid composition. The samples collected the third day were kept frozen as spare samples.

Total lipids were extracted by a modified method of (Folch et al., 1957). In brief, 20 mL of chloroform (Merck, Darmstad, Germany) and methanol (Merck, Darmstad, Germany) (2:1) were added to 1 mL of milk. The samples were centrifuged at 2000 rpm for 10 min after addition of 4 mL of 0.9% NaCl in distilled, filtered water. The lower fraction containing the lipids was collected, and the solvent was evaporated under a gentle stream of N_2. The extracted lipids were re-dissolved in hexane:chloroform:methanol (95:3:2) and loaded on a 500 mg aminopropyl column (Bond Elut, Varian, Harbor City, CA, USA) previously activated by 7 mL of hexane (Merck, Darmstad, Germany). The neutral lipids were eluted by 5 mL chloroform (Pinkart et al., 1998; Ruiz et al., 2004). The solvents were evaporated under a gentle stream of N_2. The neutral lipids were transesterified by adding 1.5 mL sodium methanolate (33.3 mg/mL) and 2 mL hexane before placing horizontally at an orbital shaker platform at 400 rpm for 30 min, according to Devle et al. (2009). The fatty acid methyl esters (FAME) in hexane were transferred to GC-vials and stored at −20 °C until analysis by GC–MS.

The FAMEs were separated and identified by using GC–MS according to Devle (2009) with some modifications. The mass spectrometer was equipped with an electron ionization ion source producing 70 eV, the mass range was m/z 40–600. The scan time used was 0.4 s and the inter scan delay time was 0.20 s. The mass spectrometer was tuned to a resolution of 1200. The ion source temperature was 200 °C and the transfer line was held at 220 °C. An Agilent 6890 Series gas chromatograph (Agilent Technology, Wilmington, DE, USA) was applied for the GC–MS combination. The column used was a 50 m CP-Sil 88 capillary column with ID 0.25 mm and 0.20 μm film thickness (Varian, Middelburgh, the Netherlands). Injections were made in split mode 1:10. The GC oven temperature was programmed from 70 °C (2 min) to 150 °C (0.5 min) at a rate of 30 °C/min, then the temperature was increased to 160 °C (14 min) at a rate of 2.0°/min, thereafter the temperature was increased to 167 °C (10 min) at a rate of 1.0 °C/min, then to 174 °C (7 min) at a rate of 7 °C/min, then 230 °C (0.5 min) with a rate of 7 °C/min and finally to 240 °C at 50 °C/min and held for 0.5 min. The total run time was 60 min. The injection temperature was 250 °C and the injection volume 1.0. Identification was performed by comparing retention times with a 37-component FAME mix (Food Industry Fame Mix, Restek, Bellefonte, PA, USA) in addition to MS library search. The individual FAMEs were expressed in relative amounts of total FAME.

2.3.2. Feed intake, sampling and chemical analysis

The goats on pasture WR and PC were dosed with synthetic even-chain C_{32} alkane twice daily (191 mg/d per goat) for 12 d starting on day 8 after onset of forage treatment in each of the two grazing periods. Individual faecal grab samples were collected twice daily at milking during the last five days (days 15–19) of the alkane-dosing period and stored frozen at −20 °C. Before chemical analysis, the faeces samples were bulked to one sample per goat and period. Grazed plants were sampled daily during the same period as dosing C_{32} (days 8–17) by hand plucking plant parts that were observed to be grazed by the goats. Two persons, one on WR and one on PC conducted the herbage sampling simultaneously. At least 50 g fresh material of each species was collected at different occasions during each period giving 3–5 samples for each species and period. The samples were frozen at −20 °C from collection until milling, approximately 2 months, and thereafter frozen for about 2 months before analysis of alkane and 6 months before feed quality analysis. Sampling of plant species and plant part (leaf, stem, bark, inflorescence *etc.*) was based on (1) knowledge on which plant species and plant part goats prefer as observed in Norway (Mayes et al., 1994), (2) visual signs on the plants of grazing and (3) high frequency of the plant species in the pasture. Individual feed intake on pasture was calculated according to Mayes et al. (1986):

$$\mathrm{I} = \frac{F_i/F_j \times (D_j + I_c \times C_j) - I_c \times C_i}{H_i - F_i/F_j \times H_j}$$

where I is the herbage intake (kg DM/d); D_j is the dose rate of C_{32} alkane (191 mg/d); F_i, C_i and H_i are respective concentrations (mg/kg DM) of the odd-chain C_{31} alkane in faeces, concentrate and herbage, and F_j, C_j and H_j are respective concentrations (mg/kg DM) of C32. The faecal recovery of the dosed and plant alkane where considered to be equal.

The amount of hay offered and the refusal for each pen and period were weighed daily from days 1 to 19, and samples of offered and refused hay and of concentrate were collected daily from days 15 to 19. The daily collected samples were bulked to one sample of each feed, pen and period for chemical analyses.

Feed and plant samples were stored frozen (−20 °C) freeze dried and stored frozen again until chemical analysis. Feed and plant samples for the analysis of alkanes and long-chain alcohols were ground using a ball mill, while feed and plant samples for feed quality analysis were ground

Table 1
Chemical composition (g/kg DM) of the forages (PC = cultivated pasture, WR = woodland rangeland, HH = high quality hay, HL = low quality hay) as consumed ($n = 2$) in early (EGS) and late (LGS) grazing season and of concentrate ($n = 6$). For concentrate standard deviation is reported in brackets.

	EGS				LGS				SEM[a]	Concentrate
	PC	WR	HH	HL	PC	WR	HH	HL		
Organic matter	940b	944b	921c	948a	939b	943b	920c	949a	1.0	922 (3.7)
Crude protein	175b	138c	198a	112d	171b	104d	198a	111d	1.7	191 (2.8)
Crude fat	44a	46a	29c	21d	34b	44a	29c	20d	0.9	51 (3.6)
Starch	–	–	–	–	–	–	–	–		286 (3.8)
NDF[b]	368d	356d	562b	642a	411c	356d	562b	643a	4.6	278 (15.9)
ADF[c]	193e	237c	268b	357a	214d	212d	269b	357a	2.8	115 (9.0)
IVDMD[d]	929a	809c	830bc	682e	844b	748d	830bc	683e	4.5	862 (27.1)
NDFD[e], g/kg NDF	794a	502d	706b	504d	632c	292e	705b	507d	6.7	435 (96.9)
NEL[f], MJ/kg DM	7.19a	6.97b	5.57e	4.66f	6.65c	6.46d	5.56e	4.66f	0.023	7.52 (0.10)
Fatty acids[g]										
C14	1.22	0.05	0.80	1.10	1.71	0.60	0.80	1.10		0.0
C16	3.63	4.15	3.40	1.80	3.81	2.73	3.40	1.80		5.6
C18:0	0.28	0.30	0.20	0.10	0.30	0.26	0.20	0.10		0.5
C18:1 c9	1.47	0.74	0.50	0.20	0.70	0.41	0.50	0.20		8.0
C18:2 c9,12	5.00	4.31	2.80	1.20	4.49	2.15	2.80	1.20		11.3
C18:3c9,12,15	16.1	14.7	10.0	3.30	18.5	9.91	10.0	3.30		1.3

Means within a row with different letters (a–f) differ significantly ($P < 0.05$).
[a] SEM = standard error of the mean.
[b] NDF = neutral detergent fibre.
[c] ADF = acid detergent fibre.
[d] IVDMD = *in vitro* dry matter digestibility.
[e] NDFD = neutral detergent fibre digestibility.
[f] NEL = net energy for lactation.
[g] Number of samples analysed were 1 for concentrate and hay qualities.

using a Tecator Cyclotec grinder (1 mm screen). The faecal samples were dried at 60 °C for 24 h and ground with a coffee grinder.

Feed and pasture samples were analysed at the Dairy One, Inc. Forage Testing Laboratory (Ithaca, NY) with wet chemical procedures. Ash content was determined using AOAC Method 942.05, crude protein by AOAC Method 990.03 and crude fat (CF) by AOAC Method 2003.05 (AOAC, 1990). Heat-stable, α-amylase-treated, sodium sulfite NDF was determined using an ANKOM fibre analyser (ANKOM Technology Corporation, Fairport, NY) based on procedures describe by Van Soest et al. (1991). Digestibility of DM and NDF was determined *in vitro* after incubation for 48 h using the ANKOM DaisyII Filter Bag Technique, ANKOM Technology, Macedon, NY. The NEL 3× maintenance was predicted from total digestible nutrients according to the NRC (2001). The content of alkanes and long-chain alcohols in feed, plant and faecal samples were analysed at the James Hutton Institute, UK, according to procedure described by (Dove and Mayes, 2006). The fatty acids in feed and plant samples were extracted according to Browse et al. (1986), and with 10 μg/sample heptadecanoic acid as internal standard (Fluka 51610). The FAME in extracts was separated and quantified by gas chromatography coupled to a flame-ionization detector as described by Mæhre et al. (2013).

2.4. Tracking of goats on rangeland with global position system (GPS)

The goats grazing on rangeland (WR) were supplied with global position system (GPS collars; Radiobjella, Telespor AS, Tromsø, Norway) that were set to record the position at 15 min intervals from days 4 to 19 in each in each period. Observations of positions of two goats in each grazing season (EGS and LGS) were omitted due to missing or very few observations, leaving GPS positions from 8 goats for 16 d within each period (EGS and LGS). On average we were left with 85.3 and 86.0 positions per goat and day in EGS and LGS, respectively, which is 89 and 90%, respectively, of the maximum daily achievable positions, respectively. Latitude, longitude and altitude data were used to estimate daily horizontal and vertical walking distances.

2.5. Calculations

Daily intake of hay per goat and pen was calculated as kg DM offered per pen – kg DM refused per pen divided by the number of goats per pen. Feed quality of consumed hay was calculated as the difference between quantity of the quality parameter offered (*e.g.* CP) and quantity of the quality parameter refused divided by the DM consumed. The quality of the herbage consumed on pasture was calculated as the weighted mean of the nutritive value of each botanical component of the diet. Least-squares optimization using available alkane and alcohols was used to obtain estimates of the botanical composition of the diet. The feed quality of the forages as consumed and of concentrate is presented in Table 1.

Energy balance was calculated as NEL intake less NEL for maintenance, milk production, live weight gain and locomotion using the energy requirement equations developed by INRA (INRA, 2010). Energy requirement for milk production (NEL, MJ/day) was estimated as milk yield (kg/day) × (2.848 + 0.0392 × (milk fat (g/kg) − 35) + 0.0235 × (milk protein (g/kg) − 31)), for maintenance (NEL, MJ/day) as 0.273 MJ per kg body weight (BW)$^{0.75}$. Energy requirement (NEL, MJ/day) per kg live weight gain and loss were calculated as 27.8 × kg BW gain and 26.3 × kg BW loss, respectively. Energy spent on physical activity (NEL/day) was calculated as 0.214 MJ/km horizontal, 1.566 MJ/km uphill and 0.712 MJ/km downhill walking.

2.6. Statistical analysis

Statistical analyses on animal performance were carried out using the software SAS 9.2 (SAS, 2009). For milk yield, and milk constituents the following mixed model procedure (method = reml) was used.

$$\text{Model 1}: \quad y_{ijklm} = m + a_i + b_j + (a \times b)_{ij} + c_k + (a \times c)_{ik} + (b \times c)_{jk} + (a \times b \times c)_{ijk} + d_l(ij) + e_m(ijl) + e_{ijklm}$$

where y is milk yield and milk quality parameters, μ is the overall mean, a_i is the fixed effect of dietary treatment, $i = 1, 2, 3, 4$ (PC, WR, HH, and HL); b_j is the fixed effect of effect of season, $j = 1, 2$ (EGS and LGS); and c_k is the fixed effect of period, $k = 1, 2$ (1 = week before allocation to dietary treatment and 2 = forage treatment period), $d_l(_{ij})$ = random effect of pen within season and forage treatment, $e_m(_{ijl})$ is the random effect of goat within season, forage treatment and pen, and ε_{ijklm} is the residual error. Effect of genotype and lactation number was also included as fixed effects (not shown in the model). The co-variation within animals was accounted for in an analysis of repeated measures. The optimal covariance structure was assessed for each parameter with attention to Akaike's and Schwarz's Bayesian criterion (Littell et al., 1998).

Table 2
Daily feed intake, nutrient and energy intake, body weight change and energy balance in dairy goats as affected by pasture type (P, PC = cultivated pasture, WR = woodland rangeland) and hay quality (H, HH = high quality, HL = low quality) in early (EGS) and late (LGS) grazing season (S), $n = 2$.

Item	Season (S)	Pasture type (P)		Hay quality (H)		SEM[a]	Significance[b]				
		PC	WR	HH	HL		S	[c]PC *vs.* WR	[d]P *vs.* H	[e]S *vs.* PC *vs.* WR	[f]S *vs.* P *vs.* H
Feed intake, kg DM/d											
Forage	EGS	0.99	0.92	1.58	0.98	0.155	**	*	*	*	NS
	LGS	0.94	1.86	1.94	1.60						
Total	EGS	1.80	1.79	2.45	1.83	0.157	**	*	*	*	NS
	LGS	1.82	2.73	2.82	2.48						
Nutrient intake, g/d											
Forage NDF	EGS	364	328	891	632	74.6	**	NS	***	(*)	NS
	LGS	386	658	1094	1027						
Total NDF	EGS	590	569	1132	869	75.3	**	NS	***	(*)	NS
	LGS	630	902	1337	1271						
Total CP	EGS	329	294	480	273	22.7	*	NS	***	NS	NS
	LGS	328	362	553	344						
Total CF	EGS	85	87	91	64	5.1	**	***	*	**	NS
	LGS	77	126	102	77						
NEL, MJ/d	EGS	13.3	13	15.4	11.0	0.94	**	*	NS	*	NS
	LGS	12.9	18.6	17.5	14.1						
DM and nutrient intake, g/kg BW											
Forage DM	EGS	16.7	16.2	27.9	18.6	2.87	**	*	*	*	NS
	LGS	17.8	33.2	36.1	28.2						
Total DM	EGS	30.4	31.4	43.2	34.7	3.09	**	*	*	(*)	NS
	LGS	34.5	49	52.3	43.7						
Total NDF	EGS	10.0	10.0	20.0	16.4	1.45	**	NS	***	NS	NS
	LGS	12.0	16.2	24.8	22.4						
DM intake, g/kg $BW^{0.75}$											
Total DM	EGS	84	86	119	94	8.2	**	*	*	*	NS
	LGS	93	134	142	120						
Body weight and energy balance											
Body weight	EGS	−132	−26	−27	−110	28.6	NS	*	NS	NS	NS
gain, g/d	LGS	−103	−61	6	−59						
Body weight,	EGS	59	57	57	53	1.7	NS	NS	NS	NS	NS
kg	LGS	53	56	54	57						
Energy balance,	EGS	2.8	−4.6	2.9	2.4	1.07	***	***	**	NS	NS
MJ NEL	LGS	5.7	1.5	7.6	6.8						

[a] SEM = standard error of the mean.
[b] NS, not significant ($P > 0.1$); (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[c] Contrast cultivated pasture (PC) *vs.* rangeland (WR).
[d] Contrast grazing (P) *vs.* hay (H).
[e] Interaction contrast Season (S) *vs.* Pasture type (PC *vs.* WR).
[f] Interaction contrast Season (S) *vs.* Grazing (P) *vs.* Hay (H).

For forage quality as consumed, feed intake and energy balance, the following model was used:

$$\text{Model 2}: \quad y_{ijk} = \mu + a_i + b_j + (a \times b)_{ij} + d_l(ij) + e_{ijk}$$

where μ is the overall mean, a_i is the fixed effect of dietary treatment, $i = 1, 2, 3, 4$ (PC, WR, HH, and HL); b_j is the fixed effect of grazing season, $j = 1, 2$ (EGS and LGS); $d_{(ij)}$ is the random effect of pen within dietary treatment and season and ε_{ijk} is the residual error.

For both models, orthogonal contrasts were used to separate treatments means for period 2 (forage treatment period).

Pearson correlation coefficients (SAS, 2009) were calculated between some milk and animal parameters using average pen data. All variables were checked for normality and log transformed if required (FFA). Treatment effects were declared significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3. Results

3.1. Herbage and concentrate nutritive characteristics

The cultivated pasture (PC) had the highest herbage DM digestibility in EGS, and in LGS the DM digestibility of PC was similar to that of HH but higher than that of HL and rangeland (WR) (Table 1). HL had the lowest DM digestibility in both seasons, while the DM digestibility of the consumed WR herbage was intermediate between PC and HH in both seasons. The neutral detergent fibre digestibility (NDFD) was highest in PC of EGS and lowest in WR of LGS, but the NEL-value was on average higher in

Table 3
Daily intake of fatty acids (g/day) in dairy goats as affected by pasture type (P, PC = cultivated pasture, WR = woodland rangeland) and hay quality (H, HH = high quality, HL = low quality) in early (EGS) and late (LGS) grazing season (S), $n = 2$.

Item	Season (S)	Pasture type (P)		Hay quality (H)		SEM[a]	Significance[b]				
		PC	WR	HH	HL		S	[c]PC *vs.* WR	[d]P *vs.* H	[e]S *vs.* PC *vs.* WR	[f]S *vs.* P *vs.* H
C14	EGS	1.21	0.05	1.27	1.08	0.12	***	***	***	*	NS
	LGS	1.61	1.10	1.56	1.76						
C16	EGS	8.16	8.69	10.25	6.53	0.45	*	(*)	NS	NS	NS
	LGS	8.49	9.99	11.53	7.79						
C18	EGS	0.69	0.71	0.75	0.52	0.03	**	**	**	*	NS
	LGS	0.72	0.92	0.83	0.60						
C18:1 c9	EGS	7.97	7.62	7.74	6.99	0.11	NS	NS	*	(*)	(*)
	LGS	7.68	7.78	7.99	7.34						
C18:2 c9,12	EGS	14.1	13.8	14.2	10.8	0.41	(*)	NS	*	NS	NS
	LGS	14.1	13.9	15.4	11.8						
C18:3 c9,12,15	EGS	17.0	14.8	17.0	4.4	1.56	*	NS	*	NS	NS
	LGS	18.6	19.6	20.6	6.4						

[a] SEM = standard error of the mean.
[b] NS, not significant ($P > 0.1$); (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[c] Contrast cultivated pasture (PC) *vs.* rangeland (WR).
[d] Contrast grazing (P) *vs.* hay (H).
[e] Interaction contrast Season (S) *vs.* Pasture type (PC *vs.* WR).
[f] Interaction contrast Season (S) *vs.* Grazing (P) *vs.* Hay (H).

the grazed herbage than in both hay qualities regardless of the seasons. The CP content was higher in HH than in the other forages, and PC had higher CP content than WR and HL in both seasons. Hay had lower CF and higher fibre (NDF and ADF) content than the grazed herbage. Herbage content of the fatty acids C18:3c9,c12,c15 and C18:2c9,12 was highest in PC in both season, and the content of these two FA in WR herbage was higher than in hay in EGS but similar to HH in LGS.

3.2. Feed intake and body weight change

Forage and total DM intake expressed in kg DM/day and in g per kg BW was on average higher ($P < 0.01$) in LGS (forage: 1.59 kg/d and 28.8 g/kg BW) than in EGS (1.12 kg/d and 19.9 g/kg BW), higher ($P < 0.05$) on WR (1.39 kg/d and 24.7 g/kg BW) than on PC (0.97 kg/d and 17.3 g/kg BW) and higher ($P < 0.05$) on hay (1.53 kg/d and 27.7 g/kg BW) than on pasture (1.18 kg/d and 21.0 g/kg BW) (Table 2). However, higher intake on WR than on PC was only observed in LGS (Forage: 1.86 *vs.* 0.94 kg/d), and DM intake was similar on the two pasture types in EGS (S *vs.* PC *vs.* WR, $P < 0.05$). Intake of forage NDF in g/day reflected forage DM intake and was on average higher ($P < 0.01$) in LGS (791 g/d) than in EGS (554 g/d) and higher ($P < 0.001$) on hay (911 g/d) than on pasture (434 g/d). Expressed as g/kg BW there were small ($P = 0.18$) differences in the total NDF intake between the two pasture types, but the NDF intake was on average 74% higher on hay (20.9 g/kg BW) than on pasture (12.0 g/kg BW).

Total intake of CP was on average higher ($P < 0.05$) in LGS (397 g/d) than EGS (344 g/d), higher ($P < 0.001$) on hay (412 g/d) than on pasture (328 g/d) but similar ($P > 0.05$) on the two pastures. Total CF intake was higher ($P < 0.01$) in LGS (96 g/d) than EGS (82 g/d), higher ($P < 0.01$) on WR (126 g/d) than on PC (77 g/d) in LGS and higher ($P < 0.01$) on pasture (94 g/d) than on hay (84 g/d). Intake of the FA C18:3c9,12,15 (17.5 *vs.* 12. g/d, $P < 0.05$), C18:2 c9,12 (14.0 *vs.* 13.1 g/d, $P < 0.05$), C18:1 c9 (7.8 *vs.* 7.5 g/d, $P < 0.05$) and C18:0 (0.76 *vs.* 0.68 g/d, $P < 0.01$), was on average higher on pasture than on hay (Table 3). Intake of C18:3c9,12,15 (13.3 *vs.* 16.3 g/d, $P < 0.05$) and C18:0 (0.67 *vs.* 0.77. g/d, $P < 0.05$) was lower in EGS than in LGS.

Goats on PC lost on average more weight than goats on WR (−118 g/d *vs.* −44 g/d, $P < 0.05$). The estimated energy balance revealed that the goats on average were in positive energy balance in LGS (5.4 MJ NEL), and that they were close to balance in EGS (0.9 MJ NEL). Goats on WR had a lower ($P < 0.001$) energy balance than those in PC in both seasons (on average −1.6 *vs.* 4.3 MJ NEL), while goats on hay (4.9 MJ NEL) had on average higher ($P < 0.01$) energy balance than goats grazing (1.4 MJ NEL).

3.3. Daily milk yield and gross composition

Daily milk yield in the pre-treatment period (the week before the goats were allocated to the forage treatments) was higher ($P < 0.001$) in EGS (2.81 kg) than in LGS (1.65 kg), and milk content of fat (38.8 *vs.* 46.4 g/kg, $P < 0.001$), protein (28.1 *vs.* 29.9, $P < 0.001$) and total solids (110 *vs.* 120 g/kg, $P < 0.001$) was lower. Milk urea content was higher (8.5 *vs.* 5.9 mmol/L, $P < 0.001$) and the milk FFA content lower (0.33 *vs.* 5.9 mEq/L, $P < 0.001$) in EGS than LGS.

Goats grazing WR yielded on average less milk (1.58 *vs.* 2.15 kg/d, $P < 0.001$), energy corrected milk (ECM) (1.70 *vs.* 2.06 kg/d, $P < 0.01$), protein (51 *vs.* 70 g/d, $P < 0.001$), lactose (68 *vs.* 94 g/d, $P < 0.001$), total solids (195 *vs.* 246 g/d, $P < 0.01$) and had lower milk urea content (6.7 *vs.* 10.5 mmol/L, $P < 0.001$) than goats grazing cultivated pasture (Table 4). On the other hand, the milk content of fat (47.7 *vs.* 37.6 g/kg, $P < 0.001$) and of total solids (122 *vs.* 114 g/kg, $P < 0.01$) were higher on WR than PC, and daily

Table 4
Effect of pasture type (P, PC = cultivated pasture, WR = woodland rangeland) and hay quality (H, HH = high quality, HL = low quality) on goat milk production and constituents in early (EGS) and late (LGS) grazing season (S).

Item	Season (S)	Pasture type (P)		Hay quality (H)		SEM[a]	Significance[b]				
		PC	WR	HH	HL		S	[c]PC *vs.* WR	[d]P *vs.* H	[e]S *vs.* PC *vs.* WR	[f]S *vs.* P *vs.* H
n	EGS	10	10	10	9						
	LGS	9	10	9	10						
Milk, kg/d	EGS	2.64	1.95	2.79	2.36	0.146	***	***	NS	NS	*
	LGS	1.65	1.30	1.54	1.03						
ECM, kg/d	EGS	2.61	2.15	2.37	2.11	0.136	***	**	(*)	NS	NS
	LGS	1.50	1.25	1.35	0.93						
Fat, g/d	EGS	106	99	90	83	6.2	***	NS	**	NS	NS
	LGS	57	54	52	38						
Protein, g/d	EGS	87	62	80	68	4.1	***	***	NS	NS	NS
	LGS	53	39	48	30						
Lactose, g/d	EGS	116	84	120	104	6.4	***	***	NS	NS	*
	LGS	72	52	64	43						
Solids, g/d	EGS	310	245	289	254	15.9	***	**	NS	NS	NS
	LGS	182	145	163	111						
Fat, g/kg	EGS	40.2	51.0	32.6	36.2	2.41	NS	**	***	NS	(*)
	LGS	34.9	44.4	33.4	36.9						
Protein, g/kg	EGS	33.0	32.1	29.0	28.9	0.66	NS	NS	***	NS	(*)
	LGS	32.1	31.9	31.4	29.2						
Lactose, g/kg	EGS	44.1	42.4	42.9	44.1	0.75	NS	(*)	NS	NS	(*)
	LGS	43.8	42.8	41.5	41.6						
Solids, g/kg	EGS	117	125	104	109	2.71	NS	*	***	NS	NS
	LGS	111	119	106	108						
Urea, mmol/L	EGS	10.8	7.1	10.9	9.3	0.29	NS	***	***	NS	***
	LGS	10.1	6.3	11.8	9.8						
FFA, mEq/L	EGS	0.23	0.26	0.35	0.55	0.159	*	NS	(*)	NS	NS
	LGS	0.23	0.19	0.30	0.17						

[a] SEM = standard error of the mean.
[b] NS, not significant ($P > 0.1$); (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[c] Contrast cultivated pasture (PC) *vs.* rangeland (PR).
[d] Contrast grazing (P) *vs.* hay (H).
[e] Interaction contrast Season (S) *vs.* Pasture type (PC *vs.* WR).
[f] Interaction contrast Season (S) *vs.* Grazing (P) *vs.* Hay (H).

milk yield was similar. Milk content of protein or FFA was not affected ($P > 0.1$) by pasture type. The effect of pasture type on milk yield and milk constituents were similar in early and late grazing season.

Grazing gave on average similar milk yield but higher milk fat (42.6 *vs.* 34.8 g/kg, $P < 0.001$), protein (32.3 *vs.* 29.6 g/kg, $P < 0.001$) and total solids (118 *vs.* 107 g/kg, $P < 0.001$) content than hay feeding. Thus, grazing tended to yield more ECM (1.88 *vs.* 1.69 kg/d, $P = 0.051$) and yielded more fat (79 *vs.* 66 g/d, $P < 0.01$) than hay feeding. Goats on pasture produced milk with lower urea content (8.6 *vs.* 10.5 mmol/L, $P < 0.001$) and tended to produce milk with lower content of FFA (0.23 *vs.* 0.34 mEq/L, $P = 0.068$) than goats on hay.

Mean daily milk yield (2.44 *vs.* 1.36 kg), ECM (2.31 *vs.* 1.26 kg), fat (95 *vs.* 50 g), protein (74 *vs.* 43 g), lactose (106 *vs.* 58 g) and total solid (275 *vs.* 150 g) were higher ($P < 0.001$) in EGS than LGS (Table 4). The effect of season on milk yield and constituents depended on forage type. Goats fed hay (2.58 kg/d) yielded more milk than goats on pasture (2.30 kg/d) in EGS, while grazing (1.43 kg/d) yielded more milk than hay feeding (1.29 kg/d) in LGS (S *vs.* P *vs.* H, $P < 0.05$). Milk urea ($P < 0.001$) content was on average higher (9.0 *vs.* 8.2 Mmol/L) in EGS than LGS in goats grazing but lower (10.1 *vs.* 10.8 Mmol/L) in goats on hay diets (S *vs.* PC *vs.* WR, $P < 0.001$). The same tendencies ($P < 0.1$) were observed for milk protein and fat contents, while milk lactose content tended ($P < 0.1$) to be lower in LGS than in EGS on hay diet and remained similar between EGS and LGS on pasture. Milk FFA was higher in EGS than LGS (0.35 *vs.* 0.23, $P < 0.05$).

3.4. Milk fatty acid composition

Goats on WR yielded on average milk with lower proportion, expressed as g/kg total FAME, of medium-chain FAs C10:0 (65.5 *vs.* 80.3 g/kg, $P < 0.01$), C12:0 (29.7 *vs.* 45.9, $P < 0.0001$) and C14:0 (93.6 *vs.* 106.7, $P < 0.05$), and of C18:2c9t11 (1.2 *vs.* 1.9 g/kg, $P < 0.01$) and higher proportion of C18:0 (173.1 *vs.* 149.8 g/kg, $P < 0.01$), C18:2c9,12 (10.0 *vs.* 8.1 g/kg, $P < 0.05$) and C20:0 (2.6 *vs.* 1.2 g/kg, $P < 0.001$) than goats on PC (Table 5).

Table 5
Effect of pasture type (P, PC = cultivated pasture, WR = woodland rangeland) and hay quality (H, HH = high quality, HL = low quality) on goat milk fatty acid composition (g/kg of FAME) in early (EGS) and late (LGS) grazing season (S).

Item	Season (S)	Pasture type (P)		Hay quality (H)		SEM[a]	Significance[b]				
		PC	WR	HH	HL		S	[c]PC *vs.* WR	[d]P *vs.* H	[e]S *vs.* PC *vs.* WR	[f]S *vs.* P *vs.* H
n	EGS	10	10	10	9						
	LGS	8	10	9	10						
C4:0	EGS	6.8	7.9	7.4	7.5	0.74	***	NS	NS	NS	NS
	LGS	10.2	11.8	11.2	9.1						
C6:0	EGS	11.0	10.6	10.5	10.0	1.32	***	NS	NS	NS	NS
	LGS	22.3	20.5	25.6	20.1						
C8:0	EGS	15.8	14.2	14.9	13.1	1.05	***	(*)	NS	NS	NS
	LGS	20.9	18.9	20.6	18.2						
C10:0	EGS	64.6	54.3	66.4	53.2	5.12	***	***	NS	NS	NS
	LGS	96.0	76.7	99.2	88.2						
C12:0	EGS	34.6	25.1	35.0	26.3	2.41	***	***	*	NS	(*)
	LGS	57.2	34.3	61.3	47.4						
C14:0	EGS	97.1	84.6	112.9	91.4	4.39	***	*	***	NS	*
	LGS	116.3	102.6	150.5	129.1						
C16:0	EGS	266.0	279.6	315.1	276.9	14.61	***	NS	**	NS	(*)
	LGS	297.6	309.3	364.4	369.4						
C16:1c9	EGS	10.1	10.3	10.0	12.0	0.70	***	NS	NS	NS	NS
	LGS	4.2	3.4	2.9	4.8						
C16:0 iso	EGS	3.1	2.2	3.0	3.2	0.39	NS	(*)	(*)	NS	NS
	LGS	3.2	2.5	3.0	4.3						
C17:0	EGS	4.2	4.5	4.6	5.6	0.44	*	NS	NS	NS	*
	LGS	3.9	4.9	3.2	4.4						
C17:1 c9	EGS	1.7	2.9	2.1	3.4	0.51	***	NS	NS	NS	NS
	LGS	1.3	0.7	1.0	1.4						
C18:0	EGS	171.3	180.7	128.2	149.6	7.67	***	**	***	NS	**
	LGS	128.3	165.6	75.9	81.8						
C18:1trans[g]	EGS	7.1	6.5	4.9	8.0	1.78	***	NS	***	NS	***
	LGS	7.5	5.9	2.1	4.0						
C18:1c9	EGS	282.5	288.4	263.5	316.7	14.75	***	NS	NS	NS	NS
	LGS	210.4	221.7	166.3	203.5						
C18:1c other	EGS	6.6	6.4	4.8	5.8	1.23	NS	NS	*	NS	NS
	LGS	6.6	5.7	2.8	3.6						
C18:2 c9,12	EGS	9.5	12.0	9.5	11.2	1.41	***	*	*	NS	NS
	LGS	6.8	8.1	5.8	5.6						
C20:0	EGS	1.4	3.5	1.2	1.4	0.32	***	***	***	NS	NS
	LGS	0.9	1.7	0.7	0.9						
C18:3c9,12,15	EGS	3.3	2.3	2.5	1.5	0.37	*	NS	***	NS	NS
	LGS	2.4	2.4	1.8	0.8						
C18:2RA[h]	EGS	2.0	1.5	1.3	1.9	0.38	***	**	*	NS	NS
	LGS	1.8	1.0	0.7	1.2						
Total SFA	EGS	676.6	669.1	700.7	638.9	18.59	***	NS	NS	NS	NS
	LGS	757.1	750.3	816.0	773.3						
Total MUFA	EGS	308.5	314.7	285.7	346.2	17.12	***	NS	NS	NS	NS
	LGS	230.9	238.2	175.7	218.3						
Total PUFA	EGS	14.9	16.1	13.6	14.8	1.95	**	NS	(*)	NS	NS
	LGS	12.1	11.4	8.3	8.2						

[a] SEM = standard error of the mean.
[b] NS, not significant ($P > 0.1$); (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[c] Contrast cultivated pasture (PC) *vs.* rangeland (WR).
[d] Contrast grazing (P) *vs.* hay (H).
[e] Interaction contrast Season (S) *vs.* Pasture type (PC *vs.* WR).
[f] Interaction contrast Season (S) *vs.* Grazing (P) *vs.* Hay (H).
[g] Mainly trans-11.
[h] Mainly C18:2c9,t11. RA = rumenic acid.

Grazing resulted in lower milk proportion of medium-chain FA C12:0 (37.8 *vs.* 42.1 g/kg, $P < 0.05$), C14:0 (100.2 *vs.* 121.0 g/kg, $P < 0.001$), C16:0 (288.1 *vs.* 331.5 g/kg, $P < 0.01$) and higher proportion of C18:0 (161.5 *vs.* 108.9 g/kg, $P < 0.001$), C18:1t11 (6.7 *vs.* 4.8, $P < 0.001$) and tended to yield higher proportion of total poly-unsaturated FA (13.6 *vs.* 11.2, $P = 0.098$) than hay feeding. The difference between hay feeding and grazing was greater in LGS than EGS (*P* values for S *vs.* *P vs.* H) for C14:0 ($P < 0.05$), C18:0 ($P < 0.01$) and C18:1t11 ($P < 0.001$).

Except for a few, almost all identified milk FA were significantly affected by season. The proportion of short- and medium-chain FA C4:0 (7.4 *vs.* 10.6 g/kg), C6:0 (10.5 *vs.* 22.1 g/kg), C8:0 (14.5 *vs.* 19.7 g/kg), C10:0 (59.6 *vs.* 90.0 g/kg), C12:0 (30.2 *vs.* 50.0 g/kg), C14:0 (96.4 *vs.* 124.6 g/kg), C16:0 (284 *vs.* 335 g/kg) and the sum of saturated fatty acids (671 *vs.* 774 g/kg) was lower ($P < 0.001$) in EGS than LGS. The proportion of long-chain FA C18:0 (158 *vs.* 113 g/kg, $P < 0.001$), C18:1c9 (288 *vs.* 201 g/kg, $P < 0.001$), C18:1t11 (6.6 *vs.* 4.9 g/kg, $P < 0.001$), C18:2c9,12 (10.5 *vs.* 6.6 g/kg, $P < 0.001$), C18:2c9t11 (1.7 *vs.* 1.2 g/kg, $P < 0.001$), C18:3c9,12,15 (2.4 *vs.* 1.9 g/kg, $P < 0.05$), C20:0 (1.9 *vs.* 1.1 g/kg, $P < 0.001$) and the sum of mono- (314 *vs.* 216 g/kg) and poly-unsaturated (14.9 *vs.* 10.0 g/kg) FA were higher in EGS than LGS.

3.5. Correlations between milk and animal parameters

The calculated energy balance was strongly correlated to milk fat and FA composition and urea content (Table 6). Increasing positive energy balance was associated with low fat content (−0.79), fat yield (−0.65), high urea content (0.63), high proportion of short- (0.62) and medium- (0.83) chain FA and C16:0 (0.63) and low content of the long-chain FA C18:0 (−0.82), C18:1c9 (−0.67) and C18:2c9,12 (−0.82). Milk content of FFA was not significantly ($P > 0.05$) correlated to other milk parameters or animal parameters, like estimated energy balance.

3.6. Goat locomotion

The goats grazing on WR walked on average 2.78 km longer daily in LGS (7.95 km/goat) than goats in LGS (5.17 km/day). As the milking parlour was located at the lowest point in the terrain, the upward (2.52 and 3.89 km/goat in EGS and LGS, respectively) and downward (2.65 and 4.05 km/goat in EGS and LGS, respectively) distance walked was similar and about 50% of the total distance.

4. Discussion

This study focused on the main hypothesis that *ad libitum* access to high quality forage on cultivated pasture would yield more milk with higher content of protein and lower content of FFA than milk produced by goats grazing free range in woodland. Furthermore, we also expected that grazing season would not influence milk production and milk constituents in goats grazing cultivated pasture but would decline in goats grazing on rangeland due to decreased forage availability and quality in the latter. A

Table 6
Pearson correlation coefficients of some milk and animal parameters ($n = 16$).

	Fat	Fat yield	Log_{10} FFA	Urea	Sum C4:0–C8:0	Sum C10:0–C14:0	C16:0	C18:0	C18:1c9	C18:2c9,12	C18:3c9,12,15	C18:2c9t11
Fat yield	0.36											
Log_{10} FFA	−0.38	0.20										
Urea	−0.73***	−0.03	0.15									
C4:0–C8:0	−0.23	−0.77**	−0.29	0.10								
C10:0–C14:0	−0.54*	−0.72**	−0.20	0.55*	0.86***							
C16:0	−0.45	−0.68**	0.01	0.32	0.63***	0.76***						
C18:0	0.70**	0.69**	−0.12	−0.61*	−0.61*	−0.86***	−0.88***					
C18:1c9	0.32	0.74**	0.30	−0.28	−0.92***	−0.93***	−0.83***	0.75***				
C18:2c9,12	0.47	0.74**	0.19	−0.35	−0.84***	−0.89***	−0.83***	0.78***	0.93***			
C18:3c9,12,15	0.32	0.38	−0.21	−0.04	−0.27	−0.34	−0.60*	0.63**	0.31	0.43		
C18:2c9t11	0.11	0.37	0.08	−0.02	−0.48	−0.42	−0.68**	0.46	0.57*	0.49	0.33	
Energy balance	−0.79***	−0.65**	0.05	0.63**	0.62**	0.83***	0.63**	−0.82***	−0.67**	−0.82***	−0.45	−0.25

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

third hypothesis was that milk FA profile, due to differences in forage botanical composition and phenological development, would be affected by forage type and grazing season. Two hay qualities were included as control feeds, as hay quality does not change during the course of time like pasture. The seasonal effect was confounded by the feeding regime preceding the forage treatments, as the goats in EGS went directly from indoor silage to the forage experimental period while the goats in LGS were out on rangeland for about 50 d before they were allocated to their respective forage treatments. Daily milk yield was about 80% lower in LGS than in EGS in the week before allocation to forage treatment. Ideally, the LGS goats should also have been kept indoor on silage until start of forage treatment experiment. This was not possible due to practical reasons, like forage availability. However, our findings have considerable applied relevance. Most dairy goat farmers in Norway depend on grazing rangeland or other pastures due both to traditions and to the fact that preserved forages have been spent during the long indoor feeding season and that forages harvested and preserved during the grazing season is required for the following indoor season. Mating/kidding time is, on the other hand, easily manipulated, so delaying the kidding season in order to have goats in earlier stage of lactation in late grazing season, as done in the current experiment, is relevant. No other studies, as far as we know, have compared seasonal effects with goats in the same stage of lactation.

4.1. Effects on feed intake

Goats are more than any other domestic livestock species able to choose parts of plants with high nutritious value, like high protein content, low fibre content and high digestibility (Lu, 1988; Baumont et al., 2000). The present study confirmed this, illustrated by the low fibre content (ADF, NDF) and relatively high digestibility of the selected forages on pasture, particularly on PC, compared to the hay consumed (Table 1). The stocking rate was very low in the current study in order to allow selective feeding and maximize intake. Goats are also 'opportunistic feeders' and change their feeding behaviour according to changes in the availability of feed and their physiological stage and requirement (Fedele et al., 1993, 2002). Goats are browsers, and if available, as in WR, browse constitute 50–80% of the forages selected by goats (Silanikove et al., 2010). Browse often contain large amounts of polyphenols, like tannins, that may restrict the digestibility and utilization, as indicated in the current study by the lower fibre digestibility of the forage selected by goats on WR than on the other diets, particularly in LGS (Table 1). It is known that dietary experience modulates feeding behaviour and diet selection, and as such less experienced animals may graze considerably less than experienced animals in the same environment (Provenza et al., 2003). Changes in diet, as imposed in the current experiment, may have had an impact on the results. In addition, likely due to their high selective ability, goats have hedonic behaviour and search for feed diversity probably to maintain the rumen environment with respect to physiological and microbial parameters (Morand-Fehr, 2003). Consequently, predicting voluntary intake by goats based on traditional dietary parameters normally used for predicting intake in other ruminants is difficult (Avondo et al., 2008). Thus, the relatively great difference in DM intake observed in the current study is likely due to this complexity of interaction between animal metabolic requirement and forage properties. The average total DM intake on PC in both seasons and on WR in EGS and HL in LGS, ranging between 31 and 35 g/kg BW, was similar to levels found in the same breed fed good quality silage or hay *ad libitum* (Hussain et al., 1996; Dønnem et al., 2011c). The intake in goats fed HH and WR in LGS (50 g DM/kg BW) is considerably higher but it has been observed in other studies (Santini et al., 1992; Fedele et al., 2002), even by browsing goats in Mediterranean woodland (Decandia et al., 2008) and on mountain pasture in Norway (Eknæs and Skeie, 2006). Lower DM and NDF intake by goats on pasture than on hay is likely because the grazing goats had the opportunity to practice high degree of selectivity, as the feed offer on the pasture was high. Therefore, the goats grazing reached their nutritional requirement at lower intake level, *i.e.* dry matter intake was more governed by metabolic control of energy intake than physical fill (Santini et al., 1992). Others have also observed increased forage DM intake with increasing forage fibre content (García et al., 1995; Bonanno et al., 2008). In the present study, the CP and NDF concentrations of the total diet by goats grazing PC were on average across seasons 18 and 34%, respectively, while the corresponding figures for goats on rangeland were 16 and 32% in EGS and 13 and 33% in LGS. Except for lower content of CP in LGS on WR, the dietary CP and NDF levels were strikingly similar and very much in line with findings in free choice trials where lactating goats themselves have shown to regulate the diet CP and NDF levels to 16.5 and 34%, respectively (Avondo et al., 2008). The CP and NDF concentration of the total diet by goats fed HH, with far less possibilities to select plant parts compared to goats on pasture, was 19.6 and 47%, respectively, while those receiving the low quality hay (HL) on average consumed a diet containing 14.4% RP and 49% NDF. The results of the current study demonstrate that forage intake on pasture differs fundamentally from housed goats, and confirms that prediction of pasture intake based on herbage chemical analysis is difficult.

4.2. Effects on milk production and composition

The difference in milk yield and milk gross composition between goats on rangeland and cultivated pasture can be, to a large extent, explained by two factors, *i.e.* lower feed value of rangeland herbage as consumed and energy expenditure due to locomotion on rangeland. Based on the walking distances calculated from positions recorded by the GPS collars, we estimated that the goats spent on average 5.8 and 9.0 MJ NEL daily on locomotion in EGS and LGS, which was about 45 and 49% of their total energy intake, respectively. We do not have records of the physical activity by the goat on PC, but we think that it was negligible compared to the goats on PR as the cultivated pasture was adjacent to the milking parlour. Milk and energy corrected milk yields production were on average 27 and 17% lower, respectively, on WR than on PC. Even though the forage

quality as consumed was slightly lower on WR than on PC (Table 1), DM and net energy intake was similar or higher on rangeland than on cultivated pasture. Thus, energy requirement for locomotion was the most important factor determining differences in milk production between WR and PC.

As discussed earlier, higher milk production in EGS than in LGS can mainly be explained by the pre-experimental feeding regime. However, the response to the forage treatments on production and milk quality in general was similar in the two seasons. Goats on PC managed to maintain their milk production during the course of the experimental period in both seasons, calculated as the milk production in the experiment relative to the milk production at the start of the experimental period. The goats on HH, WR and HL reduced their daily production of energy corrected milk by 13, 19 and 26%, respectively, in EGS, while the corresponding figures in LGS was 24, 24 and 71%.

Higher content of fat and total solids in milk produced on rangeland than on cultivated pasture is a concentration effect due to lower milk yield (Santini et al., 1991). Higher milk fat content on pasture than on hay is in accordance with Morand-Fehr and Sauvant (1980) who found that the fat content in milk was higher when the dietary concentrate proportion increased in goats that had similar energy intake. In the current experiment, goats on hay and pasture had on average similar energy intake and production, but the concentrate proportion of the diet was slightly higher (0.42 *vs.* 0.36%) on the goats grazing, particularly the PC group (0.47) as a consequence of the lower forage intake on pastures. Higher milk fat content on WR than on PC and on pasture compared to hay may also be due to lower energy balance on WR and on pasture relative to hay. This is because mobilization of body fat contributes to milk fat synthesis, and there is a highly significant correlation between milk fat content and energy balance (Chilliard et al., 2003), as also seen in the current study (Table 6).

Higher milk protein content on pasture than on hay might also be due to a somewhat higher dietary concentrate to forage ratio on pasture than on hay (Morand-Fehr et al., 2007). In addition, the balance between energy and protein for rumen microbial protein synthesis and the subsequently milk protein synthesis were probably more optimal on pasture, indicated by lower milk urea content on pasture than on hay. Min et al. (2005) also found lower milk urea content in goats grazing than goats fed hay but similar milk protein content.

The content of FFA in milk was in general low and at an acceptable level with respect to sensory attributes in both EGS and LGS for all forage types. Even in LGS, where the pasture quality was lower than in EGS, particularly on rangeland, the content of FFA was lower than in EGS. Others have found that high milk content of FFA is associated with positive energy balance, high proportion of C16:0 and low proportion of C18:0 and C18:1c9 (Dønnem et al., 2011b), while others have observed higher free fatty acid content during last part of the mountain rangeland period and has associated this with inferior herbage quality and intake (Eknæs and Skeie, 2006). We could find no association between milk FFA content and calculated energy balance or other milk quality parameters. Hence, we believe that the high content of FFA frequently observed in milk from goats grazing rangeland in Norway must have been caused by other factors, *e.g.* that the goats have reached a state where they allocate more resources to maintain body weight or gain rather than to milk production. It has been demonstrated in ruminants that long-term homeostasis is ensured by mechanisms that favour the return to the body lipid starting point after mobilization (Chilliard et al., 2000), and the activity of lipoprotein lipase, the enzyme responsible for liberating fatty acids, rises when body fat is deposited (Chilliard et al., 1987). However, the mechanism between positive energy balance, increased lipoprotein lipase activity in adipose tissue and lipolysis in milk as observed by Dønnem et al. (2011a) is still blurred, and warrant further research.

The FA composition in milk is known to be influenced by forage type (*e.g.* LeDoux et al., 2002; Chilliard and Ferlay, 2004; Chilliard et al., 2007; Sanz Sampelayo et al., 2007), and changing from hay or silage based diets to pasture is known to decrease the proportion of saturated FA and increase that of unsaturated FA (Tsiplakou et al., 2006; Chilliard et al., 2007; Renna et al., 2012a, 2012b). Many of the unsaturated fatty FA that are claimed to have positive health effects increase in milk from grazing animals, particularly C18:3c9,12,15, C18:1t11 and C18:2c9t11 (RA, rumenic acid). This effect was also observed in the present study, where goats on pasture had lower proportion of the saturated fatty acids C12:0–C16:0 and higher proportion of C18:1 t11, C18:2c9,12, C18:3c9,12,15 and RA. Higher proportion of C18:1t11 and RA in milk produced on WR than on HH could not be explained by higher intake of the precursors, C18:2c9,12 and C18:3c9,12,15, as they were similar. It may be due change in the microbial flora in the rumen of the goat due to differences in the diet (Chilliard and Ferlay, 2004) or due to plant secondary metabolites, like tannins, present in grazed woodland species that may modify rumen biohydrogenation. Grazing condensed tannins containing herbage have shown to reduce milk proportions of C18:1t11 and RA and increase proportions of C18:2c9,12 and C18:3c9,12,15 in ewes (Cabiddu et al., 2009). Similarly, the milk proportion of RA was lower and of C18:2c9,12 higher on WR than on PR in the present study. However, if this effect was due to differences in intake pf condensed tannins warrant further studies. The relatively high proportion of C18:1t11 and RA in HL, particularly compared to HH despite lower intake of precursors, has been observed in other studies as well (Chilliard et al., 2007). Although the mechanism is ambiguous, the effect is likely due to a reduced biohydrogenation of the precursor FA C18:3c9,12,15 in HL (Harstad and Steinshamn, 2010).

Different types of pastures also influence the FA profile of cow and sheep milk (Cabiddu et al., 2005; Chilliard et al., 2007). Fewer studies have been made in goats, but Di Trana et al. (2005) found that milk C18:1t11 and RA were positively correlated with the increasing intake of grass. This is in line with the current study, as the intake of the precursors C18:3c9,12,15 and C18:3c9,12 were higher, and the proportions of RA and C18:1t11 were higher in milk on the grass-dominated PC than on WR. The goats on WR had a high proportion of forbs and browse of trees and shrubs in the diet (figures not shown).

Lower proportion C8:0–C14:0 FA and higher proportion of C18:0 and C18:2c9,12 in milk produced on WR than on PC may be due to differences in energy balance. There is a strong negative correlation between energy balance and milk proportion of C18:0 (Table 6) because mobilized lipids stored in animal tissue are transferred to the mammary gland by plasma and are used in the milk synthesis (Chilliard et al., 2003). Difference in energy balance, also likely explains the difference in milk FA composition between EGS and LGS. The goats in EGS had lower energy balance than the goats in LGS, and their milk was characterized by higher proportion of FA mobilized from body fat tissues than milk produced in LGS. The milk produced in LGS, when the goats had higher energy balance, was characterized by higher proportion of *de novo* synthesized FA (C4:0–C14:0).

The study confirmed the hypothesis that goats grazing cultivated pasture yielded more milk than goats grazing on rangeland. However, the difference was not mainly due to difference in feed intake and herbage quality but due to energy spent on locomotion. We could not confirm that grazing season had no impact on milk production and quality because of confounded effects of pre-experimental diet. However, when corrected for difference in milk yield and quality at start of each period the effects of the forage treatment were similar in both periods, and the hypothesis that milk from goats grazing rangeland would have lower quality in late than in early grazing season had to be rejected. The third hypothesis, that milk FA was influenced by forage treatment and season, was confirmed.

5. Conclusion

Goats grazing heterogeneous rangeland produced 26% less milk, but the milk had higher fat and total solids content when compared to goats grazing cultivated pasture. Consequently, the difference in energy corrected milk yield was less, *i.e.* 17% less milk on rangeland than on cultivated pasture. Rangeland did not impair milk quality with respect to FFA. The effect of pasture type was similar in both early and late grazing season. Grazing resulted in milk with higher content of fat, protein, DM and lower content of urea and FFA than hay diets. The results indicate that rangeland grazing does not necessarily lead to high milk content of FFA and to increased level of FFA during the grazing season.

Conflict of interest

None.

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Paper III

Feeding a supplement rich in unsaturated fatty acids improve lipid composition and flavour in Norwegian goat milk

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ABSTRACT

The present study aimed to examine the effect of lipid supplemented concentrates on milk fat content, fatty acid composition, free fatty acids (**FFA**), lipoprotein lipase (**LPL**) activity, sensory properties and size of milk fat globules of goat milk. Thirty goats assigned to three experimental groups were fed different concentrates from 60 DIM until late lactation (230 DIM). The diets were 1) control concentrate (CONTR), 2) control concentrate added 8 % of saturated lipids (Akofeed Gigant 60; rich in C16:0) (SAT) and 3) control concentrate added 8 % of unsaturated lipids (rapeseed oil, rich in C18:1*c*9 and 18:2*c*9,12) (UNSAT).

The SAT group produced milk with the highest fat content, and the fat content was positively correlated with the mean size of milk fat globules. Goats in the UNSAT group had a higher content of the long- and unsaturated fatty acids, while milk from goats in SAT group had a higher content of palmitic and palmitoleic acid (C16:0 and C16:1). The CONTROL group produced milk with a higher content of short-, medium-, odd- and branched chain fatty acids compared to the two other groups. The content of FFA in milk were low in early and late lactation, and peaked at mid lactation (90-120 DIM). A high content of FFA were correlated with poor sensory properties (tart/rancid flavour). Goats fed the UNSAT concentrate produced a milk with a lower LPL-activity and content of FFA, better sensory properties and a higher proportion of unsaturated fatty acids.

Key Words: goat milk, fatty acid composition, FFA, milk fat globules, milk sensory properties.

INTRODUCTION

The Norwegian goat milk has previously been of variable quality both with regards to rennet coagulation properties and off-flavours, which may be a problem in the production of cheese. The problem is most prominent in mid-lactation, which sometimes coincidence with the time when the goats are let out on mountain or rangeland pasture (Eknæs et al., 2006). The off-flavours of goat milk has been found to be correlated with the content of free fatty acids (**FFA**) (Dønnem et al., 2011). FFA are a result of lipoprotein lipase (**LPL**) which hydrolyses mainly triglycerides into glycerol and FFA. The LPL is a highly potent enzyme, however it does not reach its fully lipolytic potential in milk because its substrate (the triglycerides) is localised in milk fat globules (**MFG**) surrounded by a protective membrane: the milk fat globule membrane (**MFGM**) (Deeth, 2006). In cow milk, LPL is associated with the casein micelles; however, in goat milk it is, most probably, associated with the MFGM (Chilliard et al., 2003). If the MFGM is damaged or broken, the LPL will have access to the triglycerides with excessive lipolysis as a consequence. Previous studies (Eknæs et al., 2009) have shown that extra supplements of saturated fat (C16:0 and C18:0) in the diet of goats improve the milk flavour. Calcium salts of palm oil derived fatty acids are now largely used for dairy energy supplements (Onetti & Grummer, 2004). However, the use of palm oil is criticized severely, both from an environmental sustainability point of view (Wilcove et al., 2013) and from a human health perspective since it increases milk palmitic acid which is not recommended (Shingfield et al., 2008). Hence, the search for a good substitute to palm oil in animal feed becomes increasingly important.

Oilseeds were introduced to Norway in the late fifties, and the land used for cultivation today is less than one percent of the total cultivated farmland (Granlund et al., 2010).

Rapeseeds could be a more sustainable substitute for palm oil. Feeding experiments with rapeseeds or rapeseed oil to dairy goats in other countries have shown a positive effect on milk quality. LPL activity and the level of FFA were reduced (Ollier et al., 2009) and contents of saturated fatty acids, especially C16:0 decreased and *trans*-C18:1 isomers, linoleic-, linolenic and conjugated linoleic acids (**CLA**) increased (Andrade & Schmidely, 2006; Gulati et al., 1997; Mir et al., 1999).

The aim of this study was to evaluate the effect of lipid supplemented concentrate on the following milk parameters: fat content, fatty acid composition, lipolysis, LPL-activity, FFA, and size of milk fat globules (MFG) Two different lipid sources were used (saturated palm oil

or unsaturated rapeseed oil). A concentrate with no added fat was used as a control feed. Milk samples from individual goats allocated to three feeding groups were collected six times throughout the lactation cycle.

MATERIALS AND METHODS

Experimental Design

Thirty Norwegian Dairy Goats kidding in February 2011 were fed a control diet with a concentrate mixture consisting of barley, rapeseed meal (Expro 00SF, AarhusKarlshamn Sweden AB, SE 374 82 Karlshamn, Sweden), soy bean meal, beet pulp, molasses and mineral/vitamin premix until 60 days in milk (**DIM**). Thereafter, the goats were assigned to three experimental groups each of 10 goats receiving three types of feed.

The diets were 1) **control** concentrate, 2) control concentrate added 8 % of **saturated** lipids from palm oil and 3) control concentrate added 8% of **unsaturated** lipids from rapeseed oil. The experimental groups 1) Control, 2) Saturated and 3) Unsaturated are further denoted CONTR, SAT and UNSAT, respectively. These abbreviations is also used to describe the milk (SAT milk etc.).

A detailed description of the experimental design is described previously (Inglingstad et al., 2015).

The goats received 0.9 kg of the experimental concentrate per day until the start of the mountain grazing season, thereafter 0.7 kg per day. The experimental concentrate mixtures were produced by The Centre for Feed Technology at the Norwegian University of Life Sciences, and the fat content and the fatty acid composition of the concentrates and the silage is shown in Table 1.

Milk Sampling

The goats were milked 6:00 in the morning and 15:30 in the evening. Because milk yield was lower in the evening, morning and evening milk was pooled in the ratio 0.6:0.4. Preparation and analysis of milk samples were performed on 3.5 days old milk stored at 4°C (84 hrs) (age of milk when processed by the dairies), unless otherwise specified. At 190 DIM, the goats were at the summer mountain pasture and due to limited laboratory facilities, the milk were transported by car 400 km to the University for preparation and analyses.

Total Content of Fat and FFA

To study the development of FFA during storage of the goat milk, samples for measurement of the content of FFA were analysed at three different time points : 1) at time zero (shortly after milking the milk was pasteurized, 63°C for 30 min, to inactivate LPL), 2) 36 hrs after milking and 3) 84 hrs after milking. The fat content was measured in 36 hrs old milk. The samples were added one tablet of Bronopol (2-bromo-2-nitropropane-1, 3-diol; D&F Inc., USA) and were kept at 4°C until analysis by FTIR/milkoscan (MilkoScan Combifoss 6500; Foss, Hillerød, Denmark).

Extraction, Separation and Analysis of Milk Lipids

The total lipids were extracted as described by Steinshamn et al. (2014) using a modified method of Folch et al. (1957). Internal standards (trinonadecanoin and trinonadecanoic acid, Larodan) dissolved in chloroform were added to the samples prior extraction.

The extracted lipids were re-dissolved in hexane:chloroform:methanol (95:3:2, v/v) and loaded on 500 mg aminopropyl cartriges (Hypersep spe, Thermo Scientific, Bellfonte PA, USA) for solid phase extraction (**SPE**). The cartriges were conditioned with 7 mL of hexane before loading. Neutral lipids (mainly triglycerides (**TG**)) were eluted by 5 mL chloroform, thereafter the free fatty acids were eluted by 5 mL 2 % acetic acid in diethyl ether. The solvents were evaporated under a gentle stream of N_2.

Transesterification and esterification of fatty acids were performed according to Devle et al. (2014), and the fatty acid methyl esters (**FAME**) in hexane were transferred to GC-vials and stored at -20°C until analysis.

The FAME were analysed with gas chromatography **(GC**) with a flame ionization detector (**FID**) as described by by Eknæs and Skeie (2006). The column used was a 50 m CP-Sil 88 capillary column with a inner diameter of 0.25 mm and 0.20 µm film thickness (Varian, Agilent Technologies, Matriks, Norway). The GC oven temperature was 60°C for 3 min, then raised with 10°C/min to 140°C and held for 1 min, thereafter raised with10°C/min to 160°C and held for 1 min, and finally raised with 2.5°C/min to 210°C and held for 20 min.. FID signals were transferred to a Total Chrom workstation for interpretation and calibration. FAME were identified by comparing retention time (**RT**) of standards from Larodan Fine Chemicals (Malmö, Sweden) and Sigma in addition to fatty acid profiles of goat milk obtained by GC-MS in a previous study (Steinshamn et al., 2014). Due to carry over of free fatty acids (mainly

C16:0 and C18:0) from polypropylene cartriges and tubes during sample preparation, these fatty acids were omitted from the results. This was only a problem in the fraction of FFA, and did not infer the TG fraction.

Measurement of LPL-activity

The LPL-activity was measured in milk sampled at 30, 60, 120, 200 and 230 DIM and kept at -20°C before analysis. LPL (EC 3.1.1.34) activity was measured using an artificial emulsion containing [3H]-triolein (Bernard et al., 2005).

Measurement of Milk Fat Globule Size and Composition of the Milk Fat Globule Membrane

The size of the milk fat globules were measured by laser light scattering (**LLS**) using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). The method used is previously described by Michalski et al. (2001), except that the 0.1 % SDS solution was replaced by MilliQ water.

The composition of phospholipids and cholesterol in the MFGM were analysed by Vitas AS, Oslo. Six milk samples (3 with high and 3 with low content of FFA) from the sampling at 90 DIM were chosen for this analysis, and only milk from goats on the SAT group was used to omit confounding factors such as lactation and feeding. The milk samples were kept at -20°C before analysis. For identification and quantification of polar lipids and sphingomyelin, samples were accurately weighted, dissolved in isopropanol, shaken and centrifuged. The supernatants were transferred to new vials. The pellets were washed with isopropanol, centrifuged, and the resulting supernatants were pooled with the first ones . The supernatants were evaporated to dryness and dissolved in a mix of chloroform:methanol:water and analysed with an Agilent 1100 normal phase liquid chromatography system using an Evaporative Light Scattering Detector (ELSD). Separation were performed on a PVC-SIL-NP 250x4.6 mm HPLC column (YMC) using hexane, isopropanol, acetonitrile, chloroform, tert-butyl methyl ether, water and acetic acid as mobile phase. Analytes were calibrated against known standards (Lipoid GMBH). Samples for quantification of cholesterol were accurately weighted and dissolved in methanol and a sodium hydroxide solution, then incubated for 60 minutes at 50°C to hydrolyse cholesteryl esters to free cholesterol and then centrifuged. Cholesterols were determined by an Agilent reversed phase liquid chromatography system using a diode array detector (DAD). Separation were performed on an Eclipse XDB-C8 150x4.6 particle size 5 µm

column from Agilent using methanol with ammonium acetate as mobile phase. Analytes were calibrated against known standard from Sigma-Aldrich.

Sensory evaluation of milk

Three panellists trained for sensory analysis of goat milk performed the sensory analysis of the goat milk samples. The analysis was done by scoring using a scale from 1-5, where 1 indicates pronounced flavour deviation and 5 indicates good milk without off-flavours.

Statistical analysis

Analysis of variance was performed using the MIXED procedure (Littell et al., 1998) of SAS (SAS, 1992). The measurements of milk were repeated several times for each goat, and appeared correlated. Therefore, this correlation was considered in the statistical model. The covariance structure of the repeated measurements was chosen by comparing potential structures using Akaike's and Schwarz's Bayesian information criterion (Wolfinger, 1996). Variance components (**VC**) covariance structure proved useful for all the milk data. The value at 60 DIM was used as a covariate. Analysis of variance with repeated measurements of the milk data was performed according to the following model:

$$Y_{ijkl} = \mu + A_i + B_j + A \times B_{(ij)} + C_k + A \times C_{(ik)} + d(A_t)\, \varepsilon_{ijkl}$$

Where μ is the intercept; A_i is the fixed effect of concentrate type, i=1, 2, 3 (CONTROL, SAT, UNSAT); B_j is the fixed effect of DIM, j=1, 2,….4 (DIM 90, 120, 200, 230); $A \times B_{(ij)}$ is the interaction between concentrate type i and DIM j; C_k is the fixed effect of genotype at the CSN1S1 locus, k=1,2 (E12-00, E12-01); $A \times C_{(ik)}$ is the interaction between concentrate type i and genotype j; $d(A_t)$ is random effect of goat within concentrate type and genotype and ε_{ijkl} represents the experimental error.

The difference between means was estimated by the calculated lsmeans. Differences were considered statistically significant when $P < 0.05$.

Principal component analysis (**PCA**) of the fatty acids composition (in TG), FFA content and composition and sensory data was conducted using The Unscrambler® X 10.3 (CAMO Process AS, Oslo, Norway). The fatty acid data were weighted by dividing each response variable by its standard deviation, while the sensory data were not weighted as the same scale was used during analysis. Full cross-validation was used to validate the data set.

RESULTS AND DISCUSSION

Fat Content and Fatty Acid Composition in Triglycerides

The fat content in milk was influenced both by DIM (P<0.0001) and type of lipid supplementation (P<0.0007). The fat content in milk was highest shortly after kidding (30 DIM) and in SAT milk (Table 2). A high fat content in early lactation is in accordance with other studies (Chilliard et al., 2003; Eknæs et al., 2006), and extra dietary fat fed to goats is also known to increase the fat content in the milk (Chilliard & Ferlay, 2004; Sanz Sampelayo et al., 2007; Tudisco et al., 2015). However, the difference in fat content (P<0.02) between the SAT and the UNSAT groups was not expected. One of the limitations of feeding fat supplements to dairy cows is the inhibition of their rumen microbial activity (Palmquist, 1984) which affects fibre digestion in the rumen (Palmquist, 1984) and probably depresses fibre intake (Khorasani et al., 1996). Furthermore, feeding a surplus of unsaturated fats to cows may cause milk fat depression (Bauman & Griinari, 2003). However, goats seem to tolerate addition of unsaturated fat well, and hence it is possible to alter their milk fatty acid composition by feeding (Chilliard et al., 2003). In total, 32 different fatty acids were identified and quantified from the TG fraction (Table 3). The most abundant fatty acids were palmitic acid (C16:0), oleic acid (C18:1*c*9) and stearic acid (C18:0). The contents ofall fatty acids were influenced by the lactation stage except arachidonic acid (C20:4). The content of saturated fatty acids (C4:0-C14:0 were highest in early and late stage of lactation, while the unsaturated fatty acids were highest in mid lactation. The influence of the different lipid supplements on the fatty acid composition is shown by principal component analysis (PCA). The PCA score plot separated the goats in three distinct clusters according to the feeding they received (Figure 1). Comparison of the loading plot (milk fatty acids) with score plot (goats) shows that goats in the CONTR group produced milk with a higher proportion of saturated fatty acids (except for C4:0) with less than 16 carbon atoms, including a higher content of odd- and branched chain fatty acids compared to the SAT and UNSAT groups. The odd- and branched chain fatty acids originates from ruminal metabolism of branched-chain amino acids, propionate and butyrate (Chilliard et al., 2003). A higher content of 16:0 and 16:1*c*9 throughout lactation characterized the SAT milk compared to the milk from the two other groups. These results are in accordance with previous results using the same source of saturated lipids (Eknæs et al., 2009) and reflects its high 16:0 content. The UNSAT milk had a higher content of C16:1*t*9 and fatty acids with 18 or more carbons, mainly 18:0 and 18:1c9. However, linoleic- (18:2*c*9,12) and linolenic acid

(C18:3*c*9,12,15) were higher in CONTR milk, and the content of arachidonic acid was not influenced by feed or DIM. This is likely due to the almost complete biohydrogenation in the rumen of linoleic and linolenic acids from dietary rapeseed oil, which are transformed into 18:0 and several *cis* or *trans* intermediates (Chilliard et al., 2007). Intake of long- and unsaturated fatty acids have been recommended for human consumption by health authorities and intergovernmental organizations (Fao, 2010), and some fatty acids are claimed to have health-promoting properties (Shingfield et al., 2008). If the milk is to be processed into products like cheese and butter, a higher content of unsaturated fatty acids may result in products with a softer texture. Different lipid supplementation to dairy goats has also shown to influence the proteolysis during cheese ripening (Inglingstad et al., 2015), which most probably may be explained by the influence of the different fatty acid profiles on the cheese ripening.

Mean Diameter of the Milk Fat Globules

The mean size of the MFG decreased during lactation from 3.46 µm at 30 DIM to 2.88 µm at 230 DIM, and a similar effect of lactation on the MFG size has been reported by others in bovine milk (Wiking et al., 2004). A decline in MFG size with increasing intake of grass (and an increase in milk yield) (Couvreur et al., 2006) and unsaturated lipids extruded linseeds (Lopez et al., 2008) was reported for cows, however no decrease in MFG size were observed when the goats were let out on pasture (at 200 DIM, Table 2) in this present study. The average size of MFG varies between breeds; smaller MFG were reported in milk from French Alpine (2.76 µm) (Attaie & Richter, 2000) and Sarda (2.73 µm) than in Saanen (3.63 µm) goats (Pisanu et al., 2013). The MFG size of Norwegian Dairy goats seems to be in between those breeds with an average of 3.2 µm at mid lactation (120 DIM). However, the above-mentioned effects of breed, diet and lactation stage may be confounded with fat content. Several authors report a positive relationship between the fat content and the mean diameter of the MFG of goats (Cebo et al., 2012), cows (Wiking et al., 2004) and buffalo (Ménard et al., 2010), a trend which is also confirmed in this study (r=0.72, n=186, P<0.0001). Polymorphism at the CSN1S1 may also influence MFG size as goats of the "strong" genotype (A/A) are reported to have larger MFG than those of the "weak" (0/0) genotype (Cebo et al., 2012), however the effect of genotype in the present study was not significant (results not shown). In bovine milk, smaller MFG displays also a less firm and more spreadable butter (Couvreur et al., 2006) and cheese (Michalski et al., 2004). However, smaller MFG increases the total surface area of the MFG

fraction, which makes the fat more susceptible for lipolysis by human pancreatic lipase (Berton et al., 2012) and maybe also by the indigenous milk LPL.

Lipoprotein Lipase Activity, Free Fatty Acids and Milk Flavour

Lipoprotein lipase activity decreased between 120 and 230 DIM (Table 2), which is in agreement with a study in cows (Chazal & Chilliard, 1986) but opposite to results obtained in another (Ahrné & Björck, 1985). Previous studies on goat milk showed low activity in early and late lactation and a high LPL-activity between 60-210 DIM (Chilliard et al., 2003). UNSAT milk had lower LPL-activity than CONTR (P=0.001 and SAT (P=0.02), which is supported by other studies also showing a lower LPL-activity when goats were fed unsaturated lipids (Bernard et al., 2009; Bernard et al., 2005). In early lactation, the content of FFA was low in general, although large variations were seen among goats (lowest 0.1 mM *vs*. highest 1.4 mM at 30 DIM). The FFA content peaked at mid-lactation (90-120 DIM, Figure 2A, in agreement with Chilliard et al. (2003)), where some goats displayed an unacceptable high content (up to 3.3 mM) of FFA in milk. However, other goats produced milk with a low content (0.1 mM) of FFA throughout the lactation, even at mid-lactation. Interestingly, milk from the UNSAT group had a remarkably lower content of FFA (0.8 mM) compared to milk from the CONTR (1.7 mM, P=0.05) and SAT (1.9 mM, P=0.02) groups at 90 DIM, when the problem with high level of FFA was most prominent (Figure 2A). The increase in the content of FFA from 0 to 84 hrs after milking is shown in Figure 3. The post-milking lipolysis was lower in UNSAT milk at 90-120 DIM, compared to SAT and CONTR milk, as previously observed (Chilliard et al., 2003; Ollier et al., 2009).

Both lactation stage and feed group influenced milk flavour. The highest scores were obtained in early and late lactation, while milk from mid-lactation received lower scores (Figure 2B). Milk samples with low flavour scores (1 and 2) were described as tart and/or rancid. The average sensory score for UNSAT milk was 4.5, which was higher than CONTR (3.3, P=0.004 and SAT (3.5, P=0.01) (Figure 3). This findings conflict with those from a previous study (Eknæs et al., 2009), where surplus of saturated fat in the diet decreased off-flavours in the milk compared to a diet enriched with sunflower oil rich in unsaturated fatty acids. However, other studies have shown that supplementation with unsaturated fat may reduce the content of LPL/FFA/goaty flavour in milk (Chilliard et al., 2003; Skjevdal, 1979). Indeed, previous studies have shown a correlation between the content of FFA in milk and off-flavours (Deeth & Fitz-Gerald, 2006), and this is also supported by this experiment (Figure 2 and 4, r= -0.84,

n= 184). Liberation of short- and medium chain fatty acids (C6:0-C10:0) is believed to be the cause of the specific goaty flavour (Brandsaeter & Abate, 1959), and maybe also tart or rancid flavours. Branched chain variants (methyl and ethyl) of caprylic acid are volatiles with very low flavour thresholds (Brennand et al., 1989), and are believed to be strong contributors to the flavours of goat milk (Chilliard et al., 2003). More than 20 of the free fatty acids were analysed in order to identify specific fatty acids responsible for the tart and rancid flavour. Fatty acid profiles of the FFA fraction in two samples with high and low content of FFA is shown in Figure 5. Samples with a high content of FFA and low flavour score had a high content of all the identified fatty acids, and most of the free fatty acids were highly correlated to off-flavours (Figure 4). Therefore, we were not able to link any specific fatty acid to either goat flavour or to specific off-flavours (tart or rancid). We were not able to identify any branched chain variants of caprylic acids in our samples.

Although the analysis of individual FFA may provide useful information, the method has a great potential of improvement. The use of polypropylene tubes is a common practice in most laboratories today, however, in contact with organic solvents, fatty acids are released from the material. We detected considerable amounts of C16:0 and C18:0 fatty acids in blank samples originating both from the tubes and the SPE cartridges. Those fatty acids are the most common fatty acids in milk and we decided to omit those from the results. We strongly recommend the use of acid washed glassware for sample preparation and frequent control of blank samples. This problem was only observed in the fraction containing the FFA, the blanks from the fraction of neutral lipids (triglycerides) were free from contaminations.

Some goats produced milk with a high (above 0.8 mM) content of FFA throughout the whole lactation period, while others had a high content only in mid lactation but acceptable levels at the start and end of the lactation period. On the other hand, there are goats that constantly produced milk with a low content of FFA and received high scores for flavour. We know from previous studies that there is a genetic factor (of the CSN1S1-locus) linked to high content of FFA in the milk of Norwegian Dairy goats (Dagnachew et al., 2011; Dagnachew & Ådnøy, 2014), however, goats of other genotypes than E12-00 may also have high content of FFA and off-flavours in their milk. Milk LPL activity and FFA content are reported to be higher in French goats of “weak” genotypes of CSN1S1 (Chilliard et al., 2003), however, no correlation was found in the present study between the LPL activity and content of FFA in milk (r=-0.187, n=155) nor to the genotype of CSN1S1 (results not shown).

Composition of Phospholipids and Cholesterol of the MFGM in Selected Samples

As differences in LPL-activity cannot explain the increased FFA content in some samples, we hypothesize that substrate availability may explain the differences in FFA content among the milk samples. The MFGM protects the triglycerides from lipolytic degradation, and the stability of the MFGM may depend on it composition. We therefore examined possible differences in phospholipid and cholesterol composition of the MFGM to find a possible explanation of the different degree of lipolysis in the milk samples. Examination of the phospholipid composition and cholesterol content in milk of six goats of the SAT group at 90 DIM revealed differences between milk with a high content of FFA (2.9 mM, +/- 1.25) versus those with a low content of FFA (0.5 mM, +/- 0.17) (Figure 6). In addition, those with a high content of FFA had a higher content of cholesterol (14.2 mg/100 g (+/- 1.22)) compared to those with low content of FFA (11.7 mg/100 g (+/- 1.79)). Cholesterol, together with sphingomyelin, are the major constituents of lipid rafts in MFGM; structures involved in different cellular processes, and cholesterol is reported to affect the MFGM organization (Murthy et al., 2015). The content of a hydrolysed variant of phosphatidyl choline (PC), lyso phosphatidyl choline (LPC) (also called lysolecithin), were found in remarkably higher concentration in samples with a high content of FFA (21.4 vs. 7.5 mg/100 g milk). Lysophospholipids are known to have a strong affinity for both LPL and lipoproteins and may aid the LPL by disruption of the MFGM (Deeth & Fitz-Gerald, 1983). Sundheim et al. (1983) showed that exogenous LPC enhances cow milk lipolysis when activated by blood serum but not in milk without serum addition.

The goat milk samples with a high degree of lipolysis also displayed a low content of phosphatidyletanolamine (PE). Phosphatidylinositol (PI) and phosphatidylserine (PS) were not detected in any of the samples.

CONCLUSION

This study showed that it is possible to alter the milk fat content and composition, and that feeding goats with a concentrate supplemented with unsaturated lipids increased the content of most unsaturated fatty acids and decreased the content of saturated fatty acids in goat milk. In addition, unsaturated fat supplements gave lower LPL-activity in the milk, lower content of FFA and a higher flavour score compared to milk from goats receiving saturated fat supplements or goats receiving a diet without fat supplements. Both saturated and unsaturated

supplemented feeds increased the fat content in the milk, which correlates positively with the milk fat globule size. The presence of off-flavours and the total content of FFA in milk were highly correlated, and the FFA content obtained from the routine analysis (Milkoscan) therefore provides a good proxy of the sensory properties of the milk. The positive effect of inclusion of unsaturated lipids in the diet of goats gives good promises for development of new feeding strategies with feed based on more sustainable produced lipid sources, like rapeseed oil.

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Table 1. Total fat (%) and fatty acid composition (g/100 g of total fatty acids) of concentrates and silage.

	Concentrate			
	Control	Saturated	Unsaturated	Silage
Total fat	2.2	10.7	11	3.4
C14:0	0.3	0.7	0.1	0.5
C16:0	17.9	52.6	8.1	13.3
C16:1 *cis*9	0.3	0.1	0.3	1.5
C18:0	1.8	25.9	2.4	1.6
C18:1 *cis*9	15.1	4.5	48.9	3.1
C18:1 *cis*11	2.3	0.7	3.1	0.5
C18:2 n-6	37.9	8.0	22.9	14.8
C18:3 n-3	4.5	1.0	6.9	41.0
C20:0	0.2	0.3	0.6	0.8

Table 2. Fat content (%), size of milk fat globules (MFG, D_{43}, µm), lipoprotein lipase activity (LPL, nmol/min/ml) and flavour score in milk from goats fed a control (CONTR) feed with no extra fat, or concentrates supplemented with either saturated (SAT) or unsaturated (UNSAT) fat. In the pre-experimental period (0-60 DIM) all goats received the control concentrate.

		Pre-experimental period		Experimental period					Level of Significance		
	DIM	30[1]	60[1]	90	120	190[2]	230	SEM	Feed	DIM	Feed* DIM
Item	Feed										
Fat	Contr	5.20	4.44	3.58_{b}^{x}	3.18_{b}^{y}	3.43_{b}^{xy}	3.35_{b}^{xy}				
	Sat	5.28	4.30	4.53_{a}^{x}	3.98_{a}^{y}	4.01_{a}^{y}	3.99_{a}^{y}	0.166	***	***	ns
	Unsat	5.16	4.83	4.00_{b}^{x}	3.41_{b}^{y}	3.72_{ab}^{xz}	3.46_{b}^{yz}				
	SEM[3]	0.227	0.173								
MFG	Contr	3.39	3.24	3.03_{b}^{x}	3.02_{b}^{xy}	3.00_{b}^{xy}	2.87^{y}				
	Sat	3.51	3.32	3.41_{a}^{x}	3.30_{a}^{y}	3.22_{ab}^{y}	2.93^{z}	0.085	*	***	***
	Unsat	3.47	3.48	3.30_{b}^{x}	3.16_{ab}^{y}	3.34_{a}^{x}	2.84^{z}				
	SEM[3]	0.103	0.090								
LPL	Contr	489	496		403^{x}	386_{a}^{xy}	381_{a}^{y}				
	Sat	425	421		402^{x}	360_{b}^{y}	344_{b}^{y}	8.677	**	***	**
	Unsat	418	421		395^{x}	331_{c}^{y}	334_{b}^{y}				
	SEM[3]	6.756	9.319								
Flavour	Contr	4.67	4.20	2.77_{b}^{yz}	2.47_{b}^{z}	3.47_{b}^{y}	4.27^{x}				
	Sat	4.70	4.00	2.91_{b}^{y}	3.41_{b}^{xy}	3.71_{ab}^{xy}	3.81^{x}	0.364	**	***	**
	Unsat	4.20	3.90	4.11_{a}	4.76_{a}	4.56_{a}	4.56				
	SEM[3]	0.389	0.389								

Ns: P > 0.05, *: P<0.05, **: P<0.01, ***: P<0.001

[1]All goats received the control concentrate.

[2]Mountain pasture.

[3]Standard error of mean for the pre-experimental period.

$_{a\text{-}c}$Lsmeans within a column with different subscript letters are significantly different (P<0.05) from each other

$^{x\text{-}z}$Lsmeans within a row with different superscript letters are significantly different (P<0.05) from each other

SEM: standard error of means

Table 3. Fatty acid composition (% total FA of the TG) in milk from goats fed concentrates supplemented with either saturated (SAT) or unsaturated (UNSAT) fat, or control (CONTR) feed with no extra fat. In the pre-experimental period (0-60 DIM) all goats received a control diet.

		Pre-experimental period		Experimental period					Level of Significance			
	DIM	30[1]	60[1]	90	120	190[2]	230	SEM	Feed	DIM	Feed* DIM	CSN1S1
Item	Feed											
C4:0	Control	3.47	2.09	2.11	1.63	1.48	1.58					
	Sat	3.47	2.19	2.39	1.81	1.54	1.78	0.06	*	***	ns	ns
	Unsat	3.20	1.95	2.32	1.71	1.56	1.74					
	SEM[3]	0.173	0.249									
C6:0	Control	2.31	2.19	2.20	1.69	1.64	1.99					
	Sat	2.35	2.10	1.95	1.42	1.45	1.90	0.06	**	***	ns	ns
	Unsat	2.30	2.14	2.20	1.59	1.56	2.02					
	SEM[3]	0.101	0.249									
C8:0	Control	2.33	2.16	2.06	1.71	1.49	1.99					
	Sat	2.44	2.11	1.55	1.23	1.21	1.72	0.07	***	***	*	**
	Unsat	2.42	2.29	1.97	1.51	1.40	1.99					
	SEM[3]	0.119	0.136									
C10:0	Control	9.05	8.85	8.54	7.08	5.64	8.57					
	Sat	9.33	9.17	5.52	4.42	4.31	6.86	0.29	***	***	***	*
	Unsat	9.45	9.64	6.86	5.32	4.99	7.83					
	SEM[3]	0.557	0.566									
C11:0	Control	0.70	0.44	0.42	0.37	0.27	0.69					
	Sat	0.60	0.50	0.28	0.24	0.22	0.56	0.03	**	***	ns	ns
	Unsat	0.57	0.40	0.30	0.26	0.21	0.58					
	SEM[3]	0.061	0.054									
C12:0	Control	4.62	4.13	3.72	3.21	2.45	4.62					
	Sat	4.53	4.64	2.24	1.99	1.87	3.46	0.15	***	***	***	*
	Unsat	4.73	4.93	2.67	2.29	2.10	3.75					
	SEM[3]	0.314	0.425									
C14:0 *iso*	Control	0.18	0.14	0.12	0.13	0.08	0.15					
	Sat	0.15	0.15	0.06	0.12	0.09	0.11	0.01	*	***	*	*
	Unsat	0.13	0.13	0.10	0.12	0.09	0.13					
	SEM[3]	0.026	0.015									
C14:0	Control	11.51	12.24	12.89	11.42	9.16	14.34					
	Sat	11.50	12.53	8.69	7.77	7.43	11.37	0.29	***	***	***	ns

	Unsat	11.90	12.67	9.54	8.47	7.84	12.32					
	SEM[3]	0.489	0.462									
C14:1 *cis*-9	Control	0.14	0.17	0.15	0.17	0.09	0.37					
	Sat	0.14	0.19	0.09	0.11	0.08	0.24	0.02	***	***	**	ns
	Unsat	0.14	0.17	0.09	0.11	0.08	0.23					
	SEM[3]	0.012	0.018									
C15:0 *iso*	Control	0.31	0.34	0.32	0.38	0.30	0.28					
	Sat	0.25	0.40	0.23	0.29	0.25	0.27	0.02	***	***	*	ns
	Unsat	0.28	0.35	0.25	0.34	0.24	0.28					
	SEM[3]	0.027	0.027									
C15:0 *anteiso*	Control	0.26	0.34	0.25	0.37	0.27	0.34					
	Sat	0.28	0.34	0.17	0.25	0.21	0.28	0.02	***	***	ns	ns
	Unsat	0.23	0.34	0.19	0.29	0.23	0.31					
	SEM[3]	0.040	0.018									
C15:0	Control	0.65	0.73	0.77	0.77	0.63	0.85					
	Sat	0.62	0.75	0.55	0.54	0.52	0.71	0.02	***	***	**	ns
	Unsat	0.66	0.78	0.60	0.61	0.55	0.76					
	SEM[3]	0.036	0.044									
C16:0 *iso*	Control	0.20	0.24	0.28	0.26	0.15	0.18					
	Sat	0.20	0.23	0.18	0.19	0.12	0.14	0.01	***	***	*	ns
	Unsat	0.22	0.23	0.21	0.20	0.12	0.16					
	SEM[3]	0.016	0.017									
C16:0	Control	24.87	28.71	34.99	33.66	28.76	37.92					
	Sat	24.66	27.57	42.29	39.15	36.28	40.01	0.77	***	***	***	***
	Unsat	24.79	27.70	23.91	21.78	23.37	28.70					
	SEM[3]	0.855	0.754									
C16:1 *cis*- 9	Control	0.63	0.70	0.68	0.80	0.54	1.04					
	Sat	0.67	0.70	0.97	0.89	0.62	1.08	0.04	***	***	***	*
	Unsat	0.63	0.65	0.41	0.48	0.41	0.66					
	SEM[3]	0.065	0.048									
C16:1 *trans*-9	Control	0.16	0.43	0.29	0.31	0.41	0.20					
	Sat	0.15	0.44	0.26	0.32	0.38	0.22	0.02	***	***	**	*
	Unsat	0.12	0.41	0.40	0.44	0.41	0.29					
	SEM[3]	0.34	0.020									
C17:0 *iso*	Control	0.29	0.43	0.41	0.50	0.42	0.24					
	Sat	0.33	0.38	0.30	0.41	0.35	0.22	0.02	***	***	**	ns
	Unsat	0.32	0.39	0.43	0.55	0.38	0.25					
	SEM[3]	0.038	0.029									
C17:0 *anteiso*	Control	0.57	0.32	0.36	0.34	0.24	0.35					
	Sat	0.52	0.32	0.23	0.30	0.23	0.35	0.02	*	***	***	ns
	Unsat	0.55	0.35	0.27	0.26	0.31	0.32					

	SEM[3]	0.031	0.026									
C17:0	Control	0.97	0.94	0.86	0.92	0.96	1.01					
	Sat	1.00	0.95	0.53	0.58	0.74	0.71	0.02	***	***	**	ns
	Unsat	1.00	0.97	0.64	0.70	0.84	0.79					
	SEM[3]	0.018	0.024									
C17:1 *cis*-9	Control	0.54	0.54	0.29	0.39	0.28	0.32					
	Sat	0.55	0.54	0.23	0.32	0.25	0.28	0.02	***	***	ns	ns
	Unsat	0.55	0.47	0.20	0.27	0.21	0.26					
	SEM[3]	0.049	0.044									
C18:0	Control	9.89	9.14	7.82	8.71	15.02	4.71					
	Sat	9.20	9.61	9.45	10.65	14.18	6.13	0.46	***	***	***	**
	Unsat	9.13	9.34	15.76	17.02	19.54	9.48					
	SEM[3]	1.096	0.640									
C18:1 *cis*-9	Control	21.10	19.65	16.25	19.54	21.48	14.55					
	Sat	21.98	18.96	18.65	22.64	21.11	18.39	0.55	***	***	***	ns
	Unsat	21.43	18.50	24.39	27.93	24.88	22.28					
	SEM[3]	1.381	1.227									
C18:1 *trans*-9	Control	0.33	0.33	0.26	0.31	0.40	0.16					
	Sat	0.33	0.31	0.52	0.65	0.57	0.40	0.02	***	***	***	*
	Unsat	0.30	0.33	0.90	1.01	0.80	0.49					
	SEM[3]	0.042	0.031									
C18:1 *trans*-11	Control	1.16	1.17	0.90	1.21	1.54	0.64					
	Sat	1.17	1.31	0.56	0.72	1.25	0.58	0.09	***	***	***	*
	Unsat	1.26	1.25	1.70	1.89	2.24	1.09					
	SEM[3]	0.063	0.060									
C18:2 *cis*-9, *cis*-12	Control	1.50	1.46	1.30	1.49	2.56	1.05					
	Sat	1.48	1.34	1.00	1.19	1.96	0.86	0.06	***	***	***	ns
	Unsat	1.54	1.42	1.31	1.54	2.07	1.06					
	SEM[3]	0.066	0.082									
C18:2 *trans*-9, *trans*-12	Control	0.17	0.17	0.13	0.18	0.18	0.12					
	Sat	0.15	0.18	0.12	0.16	0.15	0.12	0.01	***	***	**	ns
	Unsat	0.17	0.17	0.26	0.34	0.27	0.28					
	SEM[3]	0.012	0.014									
C18:2 *cis*-9, *trans*-11	Control	0.58	0.60	0.54	0.77	0.64	0.59					
	Sat	0.58	0.61	0.31	0.45	0.49	0.43	0.04	***	***	*	ns
	Unsat	0.62	0.59	0.66	0.86	0.71	0.63					
	SEM[3]	0.034	0.034									
C18:3n-3	Control	0.41	0.42	0.32	0.42	0.94	0.32					
	Sat	0.47	0.44	0.21	0.30	0.67	0.24	0.02	***	***	***	ns
	Unsat	0.46	0.43	0.34	0.46	0.77	0.35					
	SEM[3]	0.049	0.023									

C20:0	Control	0.20	0.22	0.18	0.26	0.87	0.18					
	Sat	0.17	0.21	0.18	0.26	0.66	0.22	0.03	***	***	***	*
	Unsat	0.19	0.22	0.37	0.50	0.82	0.32					
	SEM[3]	0.025	0.015									
C20:1 *cis*-11	Control	0.17	0.10	0.05	0.12	0.10	0.04					
	Sat	0.15	0.15	0.07	0.11	0.12	0.06	0.01	***	***	**	ns
	Unsat	0.13	0.11	0.12	0.22	0.11	0.09					
	SEM[3]	0.044	0.025									
C20:4n-6	Control	0.19	0.11	0.10	0.13	0.13	0.09					
	Sat	0.13	0.11	0.05	0.07	0.13	0.04	0.02	ns	ns	ns	ns
	Unsat	0.15	0.12	0.13	0.11	0.10	0.07					
	SEM[3]	0.023	0.016									
C22:0	Control	0.18	0.15	0.16	0.18	0.29	0.12					
	Sat	0.14	0.15	0.12	0.13	0.24	0.08	0.01	***	***	ns	ns
	Unsat	0.15	0.12	0.18	0.23	0.29	0.14					
	SEM[3]	0.038	0.017									
MUFA	Control	24.55	23.45	18.81	22.68	24.78	17.18					
	Sat	25.40	22.96	21.29	25.69	24.30	21.13	0.59	***	***	***	ns
	Unsat	24.87	22.22	28.25	32.29	29.11	25.24					
	SEM[3]	4.79	4.26									
PUFA	Control	2.84	2.75	2.28	2.86	4.32	2.08					
	Sat	2.81	2.68	1.66	2.10	3.29	1.67	0.10	***	***	***	ns
	Unsat	2.94	2.73	2.58	3.20	3.83	2.32					
	SEM[3]	0.39	0.32									
Goat FA	Control	13.69	13.20	12.79	10.47	8.76	12.54					
	Sat	14.12	13.38	9.02	7.07	6.97	10.48	0.40	***	***	***	*
	Unsat	14.12	14.07	11.04	8.43	7.95	11.85					
	SEM[3]	2.35	2.52									

Ns: P > 0.05, *: P<0.05, **: P<0.01, ***: P<0.001

[1]All goats received the control concentrate.

[2]Mountain pasture.

[3]Standard error of mean for the pre-experimental period.

[a-c]Lsmeans within a column with different subscript letters are significantly different (P<0.05) from each other

[x-z]Lsmeans within a row with different superscript letters are significantly different (P<0.05) from each other

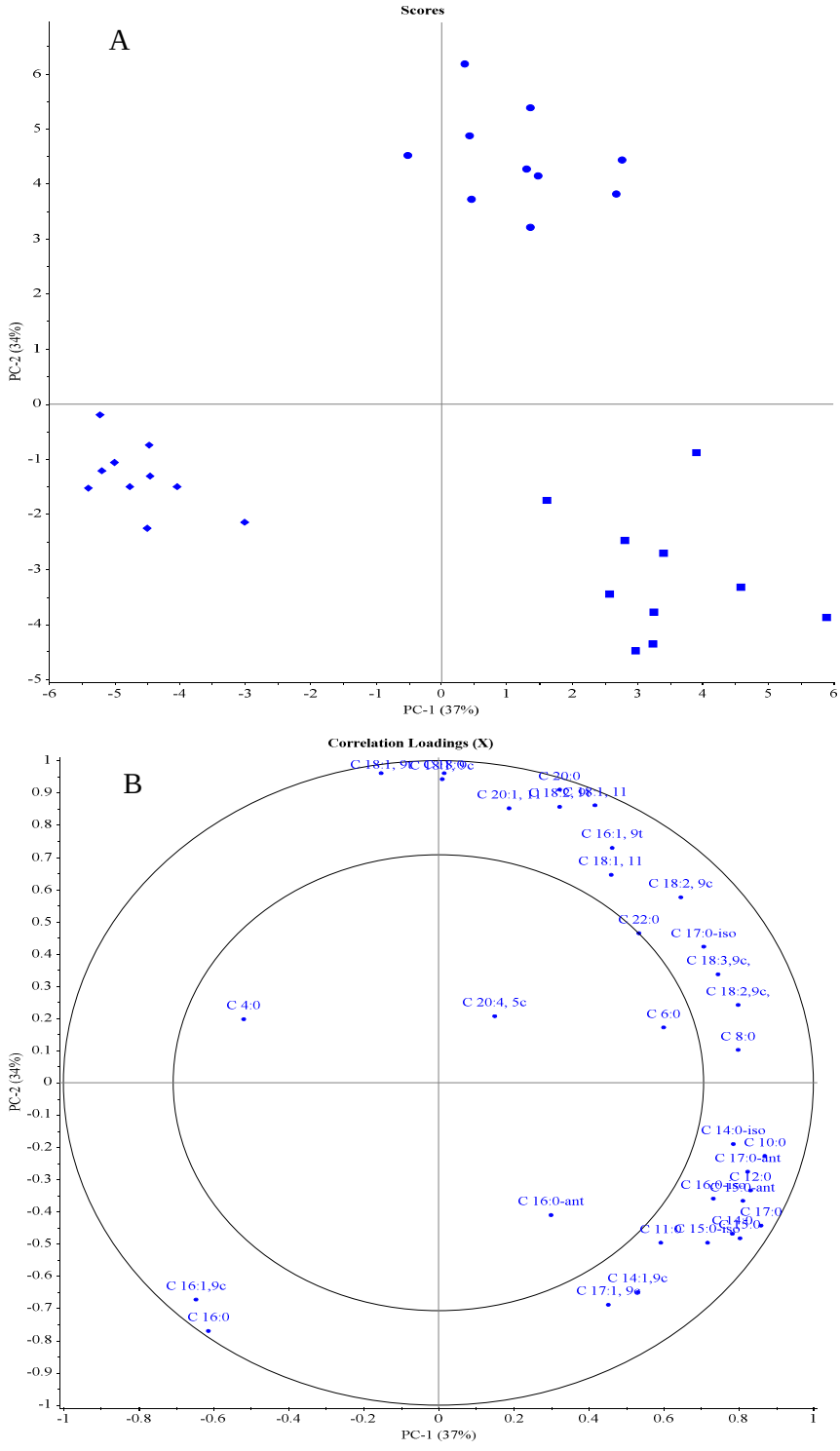
SEM: standard error of means

MUFA: Monounsaturated fatty acids

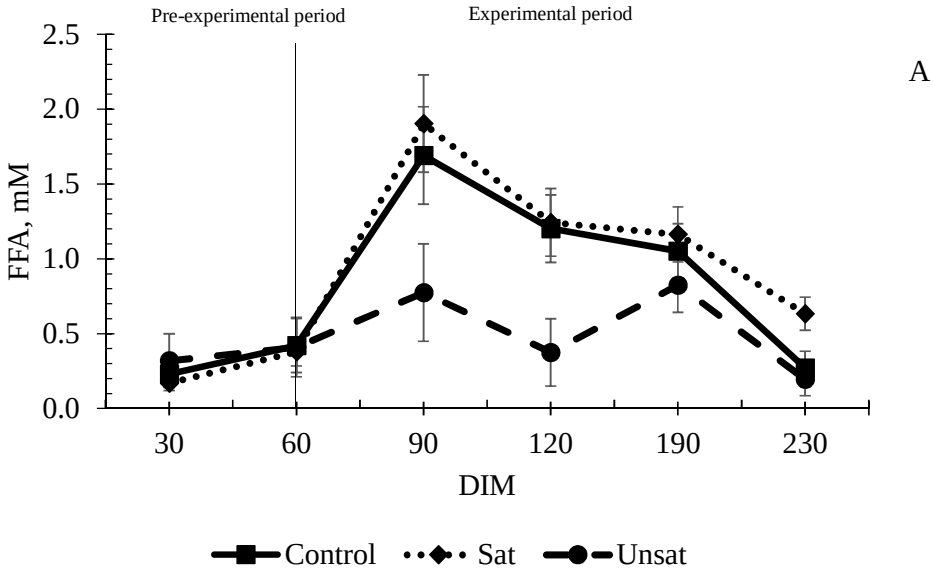
PUFA: Polyunsaturated fatty acids

Goat FA: C6:0, C8:0 and C10:0

Inglingstad. Figure 1.



Inglingstad. Figure 2



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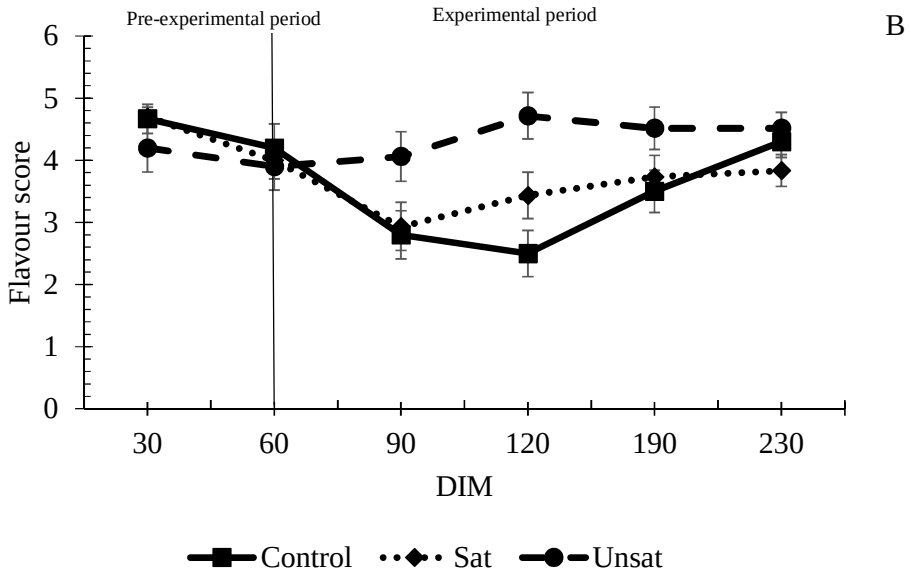
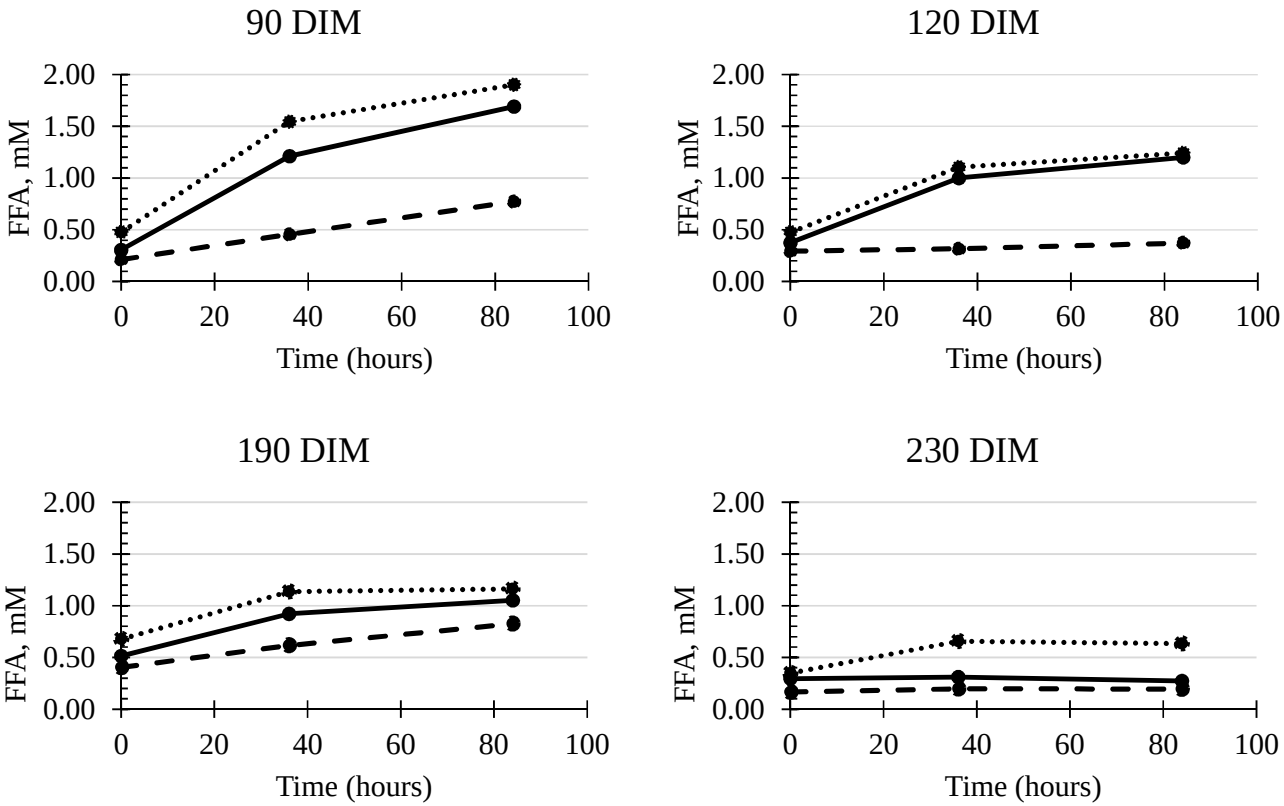


B

Inglingstad. Figure 3



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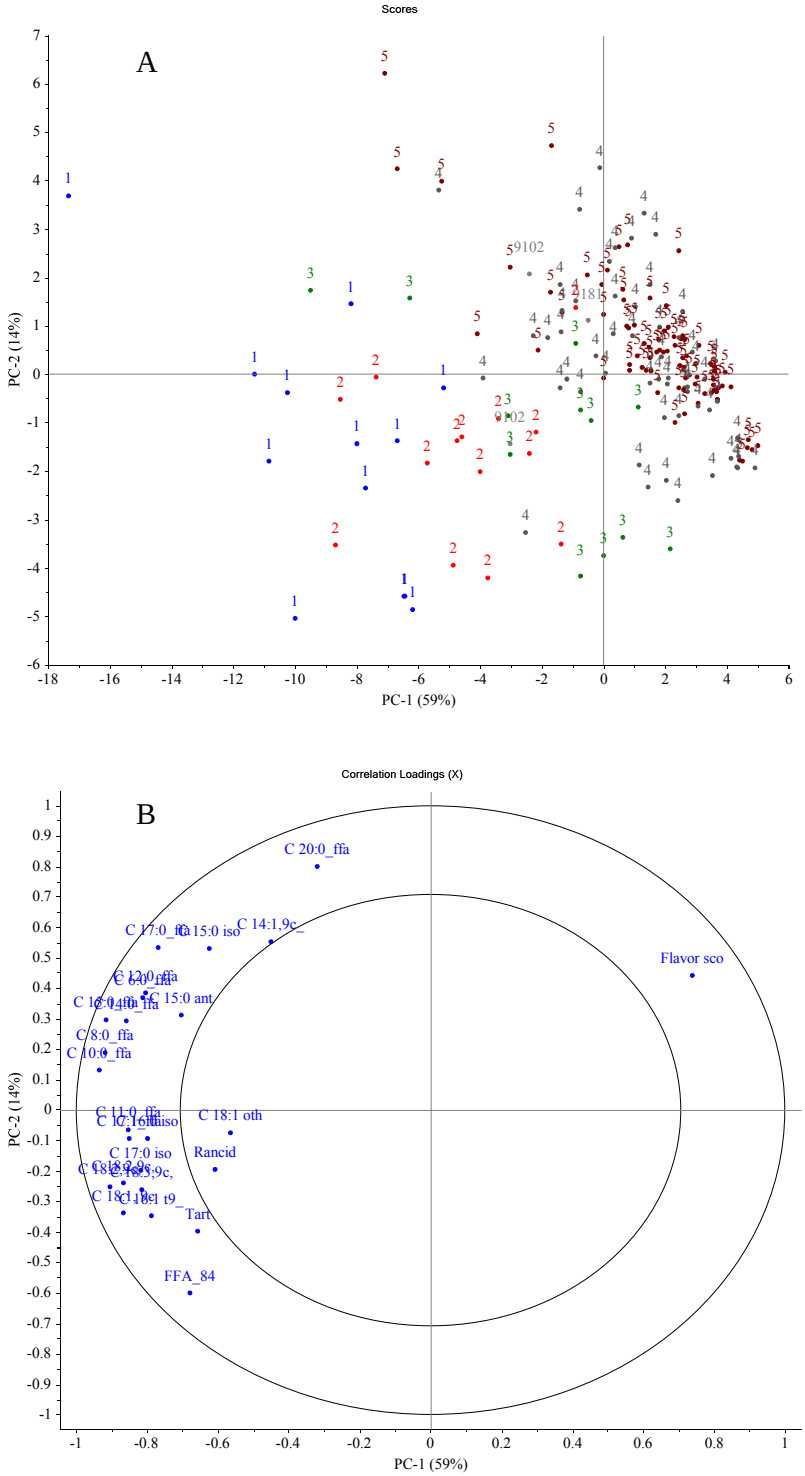

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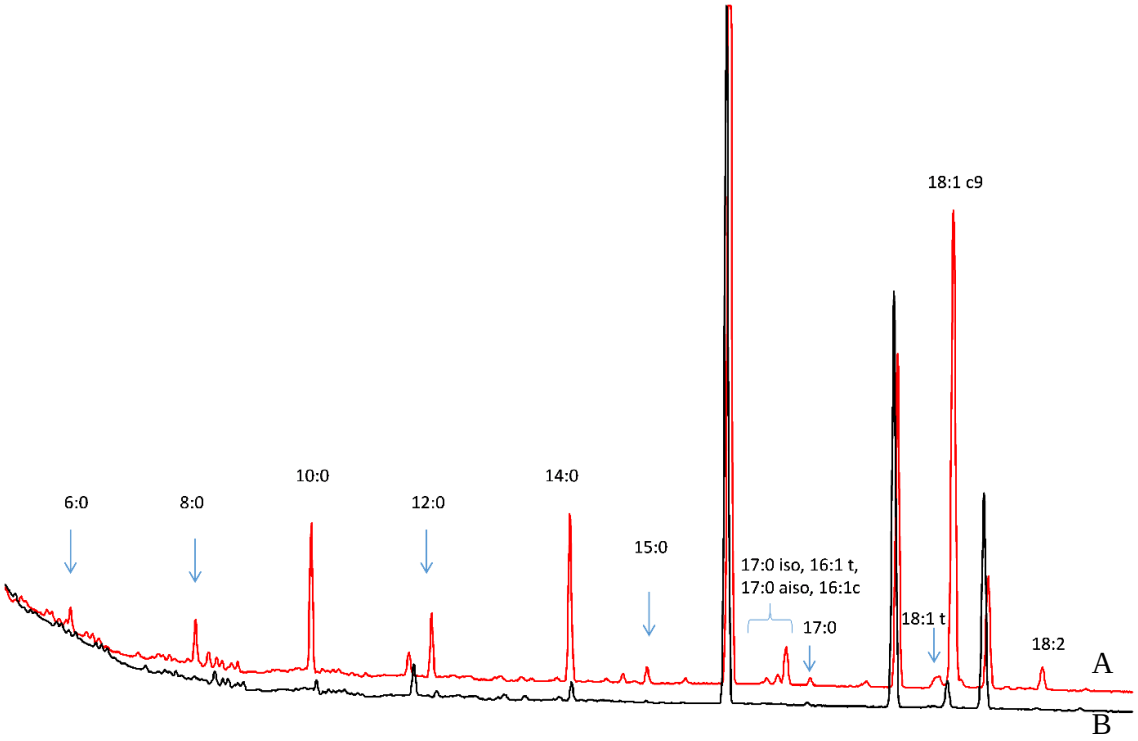

Inglingstad. Figure 6.

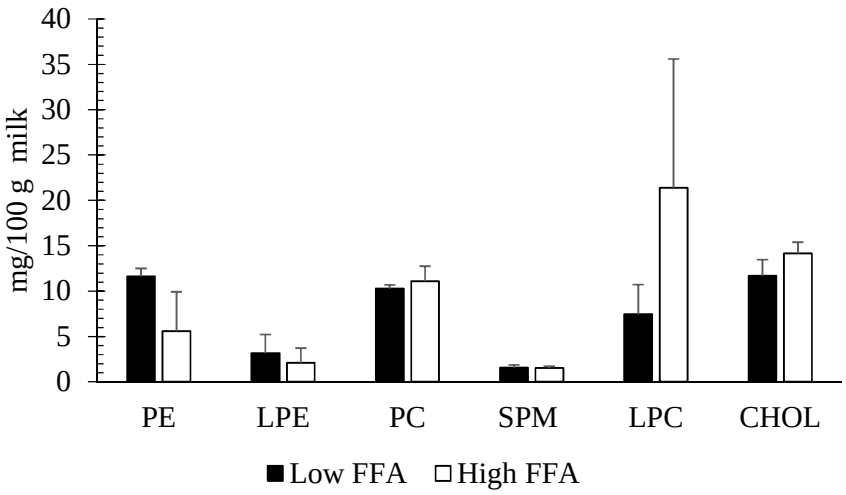

Figure captions

Figure 1. Principal component analysis (PCA) of the triglyceride (TG) fatty acid profile in milk at 90 DIM. The score plot (A) shows the distribution of the samples indicated by feeding groups (CONTR ■, SAT ♦ and UNSAT ●) and the corresponding loading plot (B) shows the distribution of variables (fatty acids). More than 70 % of the variation is explained by principal component (PC) 1 and 2. The PCA analysis revealed a similar distribution of the loadings and scores at 120-230 DIM.

Figure 2. FFA content in milk (A) and milk flavour scores (B) throughout a whole lactation from goats receiving different lipid supplemented (saturated ····, unsaturated ----, or control ──) concentrates from 61-230 DIM. The vertical line indicates start of the experimental period. Values at 30 and 60 DIM are means, 90-230 are LS-means.

Figure 3. FFA content measured in samples at 0, 36 and 84 hours, respectively in milk from goats receiving different lipid supplemented (saturated ····, unsaturated ----, or control ──) concentrates from 90-230 DIM.

Figure 4. Principal component analysis of FFA profile, total FFA and flavour scores of all samples from 30-230 DIM. The score plot (A) shows the distribution of samples indicated by their flavour score (1-5, where 5 is best (no off-flavours) and 1 indicates high degree of off-flavours) and the corresponding loading plot (B) shows the distribution of variables (fatty acids, flavours). Principal component (PC) 1 and 2 explained more than 70 % of the variation.

Figure 5. Comparison of chromatograms of the free fatty acids (FFA) in goat milk with the highest (4.3 mM, A) and lowest (0.1 mM, B) content of FFA measured at 90 DIM.

Figure 6. Content of polar lipids and cholesterol in milk from goats with high degree of lipolysis (white) and low degree of lipolysis (black). PE=Phosphtidyletanolamine, LPE=Lysophosphatidyletanolamine, PC=Phosphatidylcholine, SPM=Sphingomyelin, LPC=Lysophosphatidylcholine, CHOL=Cholesterol

Paper IV

Norwegian goat milk composition and cheese quality: The influence of lipid supplemented concentrate and lactation stage

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Abstract

In this study, milk from Norwegian goats fed a concentrate high in saturated (SAT) or unsaturated (UNSAT) lipids were compared with milk from goats fed a control concentrate (CONTROL). Milk from individual goats was sampled six times throughout the lactation (at 30, 60, 90, 120, 190 and 230 days in milk (DIM)). The milk was analysed for protein composition, renneting- and cheese making properties. Cheeses were made from milk at 90, 120 and 190 DIM. As expected, lactation stage influenced the milk composition and rennet coagulation parameters, while only minor effects of SAT and UNSAT concentrate were observed. The lipid supplemented concentrates affected cheese composition and ripening. SAT cheese had higher content of total solids and the UNSAT cheese had the lowest content of free amino acids indicating a slower ripening.

1. Introduction

In Norway, an increasing amount of goat milk is processed into cheese, hence quality aspects related to cheese production is becoming increasingly important. Cheese milk should be high in total solids; in particular casein, have a low somatic cell count (SCC), and good coagulation properties. It is known that the content of α_{s1}-casein influences the rennet coagulation properties of goat milk (Ambrosoli, di Stasio, & Mazzocco, 1988; Skeie, Inglingstad, Brunborg, & Eknæs, 2014), and the polymorphism of the gene encoding α_{s1}-casein, CSN1S1, is widely studied (Caroli et al., 2007; Martin, Ollivier-Bousquet, & Grosclaude, 1999; Ollier, Chauvet, Martin, Chilliard, & Leroux, 2008). In addition to a high frequency of a "null-allele" of CSN1S1 in the Norwegian goat population (Adnøy et al., 2003; Devold et al., 2011), flavour deviations in the milk, sometimes including a prominent rancid-tart flavour, has been a major problem (Dagnachew, Thaller, Lien, & Adnoy, 2011; Eknæs & Skeie, 2006; Skeie, 2014). Rancid and tart flavour is caused by lipolysis of the milk fat and release of free fatty acids (FFA) (Lamberet, Delacroix-Buchet, & Degas, 2000). The supply of fatty acids to the mammary gland seems to be of fundamental importance to achieve goat milk of good quality. When as much as 5% of the dry matter in the diet of goats is supplied as fat, the fat concentration in goat milk generally increases, and the lipolysis and content of FFA in milk decreases (Chilliard, Ferlay, Rouel, & Lamberet, 2003; Chilliard et al., 2007; Morand-Fehr, 2005). Eknæs et al. (2009) observed improved sensory quality of milk when increased levels of saturated long chain fatty acids (LCFA) were added to the diet of lactating goats. To improve goat milk quality, the feed industry produces concentrate mixtures supplemented with fat, mainly saturated LCFA. However, these saturated LCFA are considered as unfavourable in human nutrition. On the other hand, plant oils can be used to obtain beneficial changes in milk fat

composition by reduction in fatty acids synthesized *de novo* (C10:0-C16:0) and an increase in C18:0, cis-C18:1, conjugated linoleic acid (CLA) and polyunsaturated fatty acids (PUFA) (Bernard, Bonnet, Leroux, Shingfield, & Chilliard, 2009). There are several publications showing an effect of different lipid supplements on the fatty acid profile in goat milk (Chilliard & Ferlay, 2004; Chilliard et al., 2003; Eknæs et al., 2009). However, only a limited number of studies on how lipid feeding affects goat milk protein composition (Sanz Sampelayo, Pérez, Martín Alonso, Amigo, & Boza, 2002), cheese making properties and cheese quality (Álvarez et al., 2007; Sanz Sampelayo, Amigo, Ares, Sanz, & Boza, 1998) are available.

The objective of this study was to investigate the effect of feeding Norwegian dairy goats a saturated (SAT; rich in C16:0 and C18:0) or unsaturated (UNSAT; rich in C18:1 and C18:2) lipid based concentrate during lactation and compare those to a control (CONTROL; no extra fat) concentrate with respect to milk composition, milk coagulation properties, and sensory and chemical quality of cheese during ripening.

2. Materials and Methods

2.1 Experimental design and diets

The experiment was carried out in 2011 on 30 goats of the Norwegian Dairy Breed in their 2nd to 4th lactation. The goats kidded from February 3rd to March 7th in 2011. The experiment was performed over a whole lactation, from 0-230 days in milk (DIM). The period from 0-60 DIM was pre-experimental, where the goats were fed a control diet. At 60 DIM the goats were allocated into three experimental groups: CONTROL, SAT and UNSAT. The groups consisted of 10 goats each, balanced according to age, date of kidding, body weight and milk yield. Each of the three feeding groups consisted of seven goats heterozygous (E12-01) for the deletion in exon 12 at the α_{S1}-casein

locus, while three were homozygous (E12-00) for this deletion. From 0-130 DIM and from 200-230 DIM the goats were stabled in individual pens and received silage according to appetite (10% refusals). At 130 DIM the goats were taken to the mountain pasture. The mountain pastures were located in Folldal (62°19'N; 10°1'E), 900-1000 m above sea level (m.a.s.l.), and the goats grazed together as a flock and had free access to pasture day and night until 200 DIM. The goats received 0.9 kg of their specific concentrate per day until the start of the mountain grazing season (130 DIM), thereafter 0.7 kg per day.

The concentrate in the control feed consisted of barley, rape seed meal (Expro 00SF, AarhusKarlshamn Sweden AB, SE 374 82 Karlshamn, Sweden), soy bean meal, beet pulp, molasses and mineral/vitamin premix. The goats in the CONTROL group received this concentrate during the whole experiment. The concentrate mixtures in the groups SAT and UNSAT were based on the same mixture as the control with addition of 8 % of a source of saturated (Akofeed Gigant 60, AarhusKarlshamn Sweden AB, SE 374 82 Karlshamn, Sweden; rich in C16:0) or unsaturated (rapeseed oil (AarhusKarlshamn Sweden AB, SE 374 82 Karlshamn, Sweden), rich in C18:1 and C18:2) fat, respectively. Due to the lower fat content, the control feed had somewhat lower (7.5 MJ kg^{-1} DM) NE_L than SAT and UNSAT feeds (both 8.2 MJ kg DM^{-1}), however, the different feeds were as isoproteic as possible: 196, 191 and 195 g kg^{-1} DM for Control, SAT and UNSAT, respectively

2.2 Milk sampling and analyses

Milk was sampled from each individual goat at 30, 60, 90, 120, 190 and 230 DIM (at 90 DIM, only some analyses were performed). Milk was collected twice a day, at 6:00 in the morning and 15:30 in the evening during each sampling day in separate buckets from each goat. Evening and morning milk of each individual goat were pooled in the ratio 40:60 (1L in total), due to higher

92 milk yield in the morning. Samples were kept at 4°C until analysis (appx. 36 h), except aliquots
93 for casein composition and calcium content that were stored at -20°C. Milk samples for protein
94 analysis by the Kjeldahl method and casein micelle size were skimmed as described previously
95 (Inglingstad et al., 2014).

96 The content of lactose and somatic cells count (SCC) were analysed by Fourier Transform Infrared
97 Spectroscopy (FTIR) (MilkoScan Combifoss 6500; Foss, Hillerød, Denmark). The milk (50 mL)
98 samples for FTIR analysis were preserved with Bronopol (2-bromo-2-nitropropane-1, 3-diol; D&F
99 Control Systems Inc., San Ramon, CA). The pH, content of total protein, casein and non-protein
100 nitrogen of milk and mean size of casein micelles was measured as described by Inglingstad et al.
101 (2014). The identification and quantification of individual caseins were analysed using capillary
102 zone electrophoresis, Agilent CE (Agilent Technologies, Waldbronn, Germany) according to
103 Mestawet et al. (2014) and Skeie et al. (2014). The calcium content of milk was measured by
104 complexiometric titration with di-sodium hydrogen salt of EDTA and Erichrom black T as
105 indicator according to Visser (1976).

106 Rennet clotting properties of milk from individual goats were analysed by Formagraph
107 (Lattodinamografo, Foss Italy, Padova, Italy) according to McMahon and Brown (1982). Rennet
108 clotting time (RCT), firming time (k_{20}) and gel strength (a_{30}) were recorded as described by Skeie
109 et al. (2014). 0.08 IMCU of rennet (CHYMAX, Chr. Hansen A/S, Hørsholm, Denmark) were
110 added per mL milk.

111 *2.3 Cheese processing*

112 A semi-soft cheese (Havarti type), was produced at 90, 120 and 190 DIM. At 90 and 120 DIM two
113 replicate blocks of cheese were made at the University Pilot Plant from 50 L milk, with two days

between each replicate block. From each vat 10 cheeses (0.5 kg) were obtained. The cheese produced at 190 DIM was made at the end of the mountain pasture period, in 10 L cheese vats with only one replicate block (2 cheeses per group were obtained). Milk for the cheese produced at 190 DIM were collected from two consecutive milkings (morning and evening). For cheese production at 90 and 120 DIM, the milk used was collected over two days (four consecutive milkings), and for practical reasons cheese making were performed the week before the collection of milk for other milk samples. A starter culture CHN19 (Chr. Hansen A/S) and a *Lb. casei*, as an adjunct culture, was added into the milk. The cheese was produced as described by Skeie et al. (2014).

2.4 Cheese analysis

Samples were taken from the pasteurised milk, from cheese after 24 h and after 2 and 4 mo of ripening. A new cheese (0.5 kg) was sampled at each sampling point. Sampling for chemical and microbial analyses were performed according to IDF-standard 50C (IDF/FIL, 1995). pH, organic acids (mg g^{-1}), total solids % (TS %) and the total number of microorganisms (CFU) were analysed immediately after sampling (Skeie et al., 2014). Cheese samples for analysis of free amino acids (FAA) were grated and stored at -20°C until analysis on HPLC as described by Martinovic et al. (2013). Cheese made at 190 DIM and ripened for 4 mo were only subjected to sensory analysis due to small sample size. Sensory evaluation of the experimental cheeses ripened for two and four months were performed as described by (Skeie et al., 2014).

2.5 Statistical analysis

Analysis of variance was performed using the MIXED procedure (Littell, Henry, & Ammerman, 1998) of SAS (SAS, 1992). The measurements of milk were repeated several times for each

136 animal, and appeared correlated. Consequently, this correlation was taken into account in the
137 statistical model. The covariance structure of the repeated measurements was chosen by comparing
138 potential structures using Akaike's and Schwarz's Bayesian information criterion (Wolfinger,
139 1996). Variance components (VC) covariance structure proved useful for all the milk data. The
140 value at 60 DIM was used as a covariate. Analysis of variance with repeated measurements of the
141 milk data was performed according to the following model:

142 $Y_{ijkl} = \mu + A_i + B_j + A \times B_{(ij)} + C_k + A \times C_{(ik)} + d(A_t) \varepsilon_{ijkl}$

143 Where μ is the intercept; A_i is the fixed effect of concentrate type, i=1, 2, 3 (CONTROL, SAT,
144 UNSAT); B_j is the fixed effect of DIM, j=1, 2,….4 (DIM 90, 120, 200, 230); $A \times B_{(ij)}$ is the
145 interaction between concentrate type i and DIM j; C_k is the fixed effect of genotype at the α_{S1}-
146 casein locus, k=1,2 (E12-00, E12-01); $A \times C_{(ik)}$ is the interaction between concentrate type i and
147 genotype j; $d(A_t)$ is random effect of goat within concentrate type and genotype and $\mathcal{E}_{ijkl}$ represents
148 the experimental error. For the statistical analysis of the cheese data an autoregression covariation
149 structure proved useful for all data. Significant differences ($P<0.05$) between the fixed treatment
150 factors during cheese making and the ripening age on the dependent variables of the cheese were
151 found by an analysis of variance with repeated measurements according to the following model:
152 $Y_{ijkl} = \mu + A_i + B_j + C_k + d + \varepsilon_{ijkl}$ where μ is the intercept; A_i the fixed effect of concentrate, i=1,2,3
153 (CONTROL, SAT, UNSAT); B_j is the fixed effect of DIM, j=1,2,3 (DIM 90, 120, 200); C_k is the
154 repeated effect of ripening age, with k=1,2 (two months, four months); d is the random effect of
155 the two replicate blocks; and $\mathcal{E}_{ijkl}$ is the experimental error.

156 Partial least square regression using the FAA data as X variables and sensory scores as Y
157 variables was conducted with The Unscrambler® X (CAMO Process AS, Oslo, Norway). The
158 amino acid data were weighted by dividing each response variable by its standard deviation,

while the sensory data were not weighted as the same scale was used during analysis. A full cross-validation was used to validate the data set.

3 Results and Discussion

3.1 Milk

3.1.1 Effects of feeding

The content of non-fat solids in the goat milk were, in general, only affected to a minor extent by the different lipid supplemented concentrates. This is in accordance with previously reported results in Norwegian goats milk (Eknæs et al., 2009) for protein and lactose. However, at mid-lactation (90-120 DIM) significant effects of the lipid composition of the concentrate were found for the content of lactose ($P< 0.05$), calcium ($P<0.01$) and in pH ($P<0.01$). These parameters obtained higher values in the UNSAT milk compared to the CONTROL milk (Table 1). Such effects on milk components of unsaturated fats in the feed of goats has not been previously reported according to our knowledge. Despite a higher (but not statistically significant, $P>0.05$) SCC, the UNSAT milk had the highest gel firmness at mid-lactation. This indicates that a high SCC does not automatically give a poor cheese milk, and this is in accordance with other published results (Chen, Wang, Van Kessel, Ren, & Zeng, 2010). The content of lactose in milk has previously been found to be correlated with gel firmness during renneting (Inglingstad et al., 2014; Leitner, Merin, & Silanikove, 2011)

Studies on different protein feed sources (Sanz Sampelayo, Pérez, Gil Extremera, Boza, & Boza, 1999) and different types of pastures (Inglingstad et al., 2014) revealed that feeding may change the proportion of the individual caseins in goat milk. Sanz Samplayo et al. (2002) studied the effect of three levels of PUFA in the goat diet (0 %, 9 % and 12 %), and showed a lower content of α_s-caseins in the milk when the concentration of PUFA in the feed increased. In the present study, a significant difference ($P<0.05$) between the feeding groups was only found in the ratio of κ-casein to total casein in mid-lactation (120 DIM) where the concentration of κ-casein was higher in CONTROL (24.7%) milk than in SAT (22.5%) milk. Studies of cow milk have revealed a decrease in protein and especially casein content when the content of fat (especially unsaturated fat) was increased in the diet (Chilliard & Ferlay, 2004; Coppock & Wilks, 1991; Jenkins & Jenny, 1992). However, this was not observed in our study on goat milk. Actually the UNSAT milk had the highest protein content measured when the goats were at the mountain pasture (190 DIM) ($P<0.05$ compared to SAT). Otherwise, no significant differences in total content of protein, casein, or non-protein nitrogen (NPN) between the CONTROL, SAT or UNSAT milk were observed.

Several studies report no or few effects on rennet coagulation properties of milk by feeding different unsaturated oils (Martínez Marín et al., 2011), different forage to concentrate ratio (Mele, Serra, Rafanelli, Conte, & Secchiari, 2010; Tufarelli, Dario, & Laudadio, 2009), different protein sources (Laudadio & Tufarelli, 2010; Sanz Sampelayo et al., 1998) or different forage (Inglingstad et al., 2014). Even though feeding hardly changes the rennet coagulation properties of milk, our study showed an improved k_{20} by feeding lipid supplemented concentrates. The CONTROL milk had longer firming time (36 min) compared to SAT (26 min, $P<0.05$) and UNSAT milk (23 min, $P<0.01$) at 90 DIM. The UNSAT milk displayed the most stable values of a_{30} throughout lactation (Figure 2).

3.1.2 Effects of lactation stage

Stage of lactation is one of the many factors that influence milk composition and its technological properties, and this was also reflected on the different milk components in the current study (Table 1). The content of lactose in the milk decreased throughout the lactation period (from 4.86% at 30 DIM to 4.06% at 230 DIM, $P<0.0001$), except for a plateau at 90-120 DIM. The SCC was highest (7.2 log units mL^{-1}, $P<0.0001$) at 190 DIM, when the goats were grazing mountain pasture. Also the highest milk pH was measured when the goats were on mountain pasture, 6.80 compared to 6.59 at 60 DIM during indoor feeding. The effect of the lactation stage on pH was significant ($P<0.0001$). The contents of total protein and casein were higher in early lactation, decreased as the goats entered their mid lactation, and increased again in the late lactation period. The effect of the lactation stage was significant ($P<0.0001$) with respect to protein and casein content. The highest content of total protein (3.2%) and casein (2.5%) was measured at the first sampling (30 DIM) and then the protein content gradually decreased to its lowest level (2.5% and 1.9%, protein and casein, respectively) at 120 DIM. The protein content increased again towards the end of lactation (3.0% and 2.8% protein at 190 and 230 DIM respectively and 2.4% and 2.2% casein at 190 and 230 DIM respectively). The content of whey proteins were not influenced by lactation stage nor by the concentrate (Table 1). The highest casein to whey ratio was measured at 190 DIM (mountain pasture), and was significantly ($P<0.05$) different from 120 DIM, but not from 230 DIM. The lowest content of non-protein nitrogen (NPN) in the milk (0.04%) was measured during mountain grazing and the highest values (0.05%) were observed at the start and end of lactation, where lactation stage was highly significant ($P<0.0001$). The content of calcium decreased gradually from 1.53 g kg^{-1} at 30 DIM and 1.6 g kg^{-1} at 60 DIM to 1.3 g kg^{-1} at 230 DIM (Table 1), and the main effect of lactation stage was highly significant ($P<0.0001$). Similar trends have been reported

by Brendehaug and Abrahamsen (1986); Guo, Park, Dixon, Gilmore and Kindstedt (2004); Mestawet et al. (2014); Mestawet et al. (2012). The composition of the individual caseins is shown in Figure 1. The most abundant casein in all samples was β-casein, which constituted to more than half of the casein fraction. β-casein displayed a gradual decrease when lactation proceeded, with the lowest level found at 120 DIM (12.6 g L^{-1}). The relative values of β-casein content to total casein were lowest at the start (56% at 30 DIM) and at the very end of lactation (55.5% at 230 DIM), and highest at 190 DIM (60.6%). κ-casein, in contrast to β-casein, were highest in relative value to total casein at the start (25.5% at 30 DIM) and end of the lactation curve (27.2% at 230 DIM). The relative content of κ-casein was lowest at 200 DIM, contributing to 22.7% of the total casein. The content of α_{s1}-casein was highest at the start (11.2% and 3.5 g L^{-1} at 30 DIM) and at the end (11.4% and 2.9 g L^{-1} at 230 DIM) of lactation, while the lowest contents were measured at 120 DIM, where the relative concentration contributed with only 9.8% to the total casein and 2.2 g L^{-1} milk. In contrast to the other caseins, the content of α_{s2}-casein was not influenced by lactation stage. The relative concentration of α_{s2}-casein to total casein was quite constant at ~7.5% from 30-190 DIM, before it was reduced to 6.5% at 230 DIM ($P<0.0001$). In the current study, the content of all caseins were higher in the early lactation stage than at the later stages. This finding is different from the finding reported by Mestawet et al. (2014) in Ethiopian goats during the late lactation period. During this period, as the milk volume decreases, the milk components are expected to concentrate and hence to be higher than in the earlier stages of lactation. In this study the individual caseins were at their lowest in the mid-lactation stage (DIM 120) except for the content of α_{s2}-CN which was unaffected by lactation stage.

The micelle size was influenced by lactation stage (Table 1). The casein micelles were about 220 nm in the start of lactation (30 and 60 DIM) and smallest towards the end (210 nm at 230 DIM) of

lactation. The casein micelles were larger at mid-lactation (229 nm at 120-190 DIM) than at 230 DIM (210 nm) ($P<0.0001$). Similar effects of lactation stage is also reported by Mestawet et al. (2014) for Ethiopian goats, otherwise information on how stage of lactation affects the size distribution of casein micelles in goat milk is scarce and to the best of our knowledge, very little data has been reported. Previous studies on milk of individual cows found no correlation of casein micelle size with lactation stage, nor with the fat or protein content of the milk, age of cow or the milk volume produced (de Kruif & Huppertz, 2012).

The rennet coagulation properties of milk were improved (shorter RCT, smaller k_{20} and greater a_{30}) in early compared to late lactation. The RCT values increased sharply from 10 min at 90 DIM, to 16 min at 190 DIM (Figure 2). Highest values of a_{30} was obtained at 30, 60 and 190 DIM. The increase in curd firmness at 190 DIM when the goats were at mountain pasture (Figure 2) was somewhat unexpected as there is no obvious indications of this with regards to the milk composition. However, it is most likely that the increased protein and casein content may be the main factors.

3.1.3 Effects of Genotype

The influence of genotype at the α_{s1}-locus was not the aim of this paper, however, as we know from previous studies that genotype influences also other parameters than the content of α_{s1}-casein (Devold et al., 2011; Pierre et al., 1998; Skeie et al., 2014), it was included as a factor in our statistical model. Norwegian goats homozygous for the deletion in exon 12, E-00, had a lower total protein-, and casein content, and a lower content of individual caseins and calcium. In addition, these goats had longer RCT, longer gel firming time (k_{20}) and a weaker curd (a_{30}) compared to the heterozygous goats, E12-01.

3.2 Cheese

3.2.1 Effects of feeding

The cheese made of milk from goats fed a concentrate with saturated fat (SAT cheese) obtained a higher ($P<0.01$) total solids (TS) content than the cheese made of milk from the goats fed a concentrate of unsaturated fat (UNSAT cheese) (Table 2). The TS in cheese made of milk from goats fed the control concentrate (CONTROL cheese) did not differ significantly from the other cheeses. As the cheeses were ripened in foil, the total solids did not change significantly during the ripening period. The cheese pH was not influenced by feeding regime, however, as expected, pH increased significantly ($P<0.001$) during ripening. The fat source of the concentrate influenced the texture of the cheese, but not the flavour. The CONTROL cheeses generally obtained a better texture score than the UNSAT cheese. The sensory analyses revealed that the texture attributes of the cheeses were significantly ($P<0.05$) influenced by type of concentrate (Table 2). The texture of the UNSAT cheese obtained a lower score ($P<0.05$) than the CONTROL cheese and the UNSAT cheese was also more doughy ($P<0.01$) than the CONTROL and the SAT cheese. The texture, taste and flavour of the cheeses did not improve during ripening, as after 2 mo of ripening the main scores were higher ($P<0.05$, effects 0.3, 0.6 and 0.4, respectively), and the cheese were less doughy ($P<0.01$, effect -0.5) than after 4 mo of ripening.

During cheese ripening, the caseins are degraded by enzymes from rennet, lactic acid bacteria (LAB) and indigenous enzymes (i.e. plasmin). Therefore the content of FAA in the cheese can be used as a ripening index (Fox, Guinee, Cogan, & McSweeney, 2000). The proteolysis is mainly responsible for the textural changes and flavour development during ripening. The content of FAA increased ($P<0.001$) in all cheeses during ripening (from 2 to 4 mo), and the increase of FAA from 2 to 4 mo were higher in the CONTROL and SAT cheeses than in the UNSAT cheeses (Figure

293 3a). The ripened SAT cheese had higher levels (65.5 µmol g^{-1} cheese) ($P<0.05$) of all FAA except
294 for gamma-aminobutyric acid (GABA), than UNSAT (50 µmol g^{-1} cheese) and CONTROL (55
295 µmol g^{-1} cheese) cheese. The CONTROL cheese had higher (0.14 µmol g^{-1} cheese) ($P<0.01$)
296 amounts of GABA than the UNSAT cheese (0.1 µmol g^{-1} cheese). Due to the slower increase of
297 FAA, the UNSAT cheese therefore appeared to have a slower proteolysis than the CONTROL and
298 the SAT cheese (Figure 3a). The development of FAA are influenced by factors like moisture
299 content, pH and the content of proteolytic enzymes. Generally, a lower proteolytic activity is
300 obtained as the moisture content decreases in cheese. However, the UNSAT cheese had the highest
301 moisture content of the cheeses (Table 2), and a slower proteolysis was not expected. It is unlikely
302 that the UNSAT cheese had a higher content of ripening enzymes derived from the starter culture,
303 as the highest content of *Lc.* spp throughout ripening was found in the CONTROL cheese ($P<0.05$),
304 with SAT and UNSAT cheese having a similar, but somewhat lower content (Table 2). The content
305 of Lactobacilli did not differ between the cheeses (results not shown). The pH in the different
306 cheeses was similar and should not facilitate differences in proteolytic activity between the
307 cheeses. Differences in moisture, pH and ripening enzymes from lactic acid bacteria (LAB) can
308 therefore not explain the differences in the content of FAA in the experimental cheeses.

309 *3.2.2 Effects of lactation stage*

310 Cheese produced at 190 DIM had a higher content of TS ($P<0.001$) and a higher pH ($P<0.001$)
311 than cheese made at 90 and 120 DIM. This can be attributed to differences during cheese
312 production, as the cheese produced at 190 DIM was made on the mountain summer farm. The
313 summer farm is located 6 h drive from the dairy pilot plant with no transport possibilities to the
314 university pilot plant. The cheeses were therefore made at the summer farm in small 10 L cheese
315 vats, which obviously made it somewhat difficult to replicate the cheese made at 90 and 120 DIM.

However, as described above, a higher curd firmness (a_{30}) were obtained at this lactation stage (190 DIM) compared to 90 and 120 DIM (Figure 2).

All the FAA except of Arg and GABA were influenced ($P<0.05$) by DIM (Figure 3a). Ripened cheese produced at 90 DIM had lower content of FAA than cheese produced at 120 and 190 DIM. The content of all FAA except for GABA increased ($P<0.05$) during cheese ripening. The higher content of FAA in cheese produced at 120 and 190 DIM indicates higher proteolysis in those cheeses. Hence, proteolytic activity may be dependent upon lactation and it is known that the plasmin activity in goat milk is higher in late lactation (Cortellino, Locci, & Rampilli, 2006; Fantuz, Polidori, Cheli, & Baldi, 2001). The development of most FAA during ripening was similar; however, the development of GABA, Tyr, Arg and Trp differed somewhat from the others. Tyr was highest ($P<0.001$) in cheese made from 120 DIM (Figure 3b), while the development of Arg was not influenced by DIM (results not shown). During ripening the content of Trp was higher ($P<0.001$) and increased more during ripening in cheese made at 120 and 190 DIM (Figure 3c) than cheese made at 90 DIM. Plasmin and rennet hydrolyse the most abundant proteins, α_{S1}-CN and β-CN where they split specifically at Trp and Tyr positions (Upadhyay, McSweeney, Magboul, & Fox, 2004). Moreover, it has been shown that in goat milk cheeses, the α_S-CNs are the most extensively degraded CNs (Hayaloglu, Tolu, & Yasar, 2013; Tejada, Abellán, Cayuela, Martínez-Cacha, & Fernández-Salguero, 2008).

Cheeses produced at 190 DIM obtained a better texture score ($P<0.001$) than cheese produced at 90 and 120 DIM. This is most probably connected to the differences in TS, and a higher TS seems to be beneficial for the cheese variety made in the present experiment.

3.3.3 Multivariate analysis of free amino acids and sensory data

Partial least square (PLS) regression analysis revealed that texture properties were influenced by the content of FAA (Figure 4). Factor 1 and factor 2 separated the cheeses according to their content of FAA and their texture properties, respectively. All UNSAT cheeses were doughy and had a poor texture, except UNSAT cheese made at mountain pasture (190 DIM). In addition, the UNSAT cheeses had a low content of FAA (Figure 3) which means that these cheeses had a less pronounced proteolysis. Cheeses ripened for 4 mo (in the ellipse in Figure 4b) had a higher content of FAA, as expected. However, the SAT cheeses appeared more ripened (higher content of FAA) than the UNSAT and the CONTROL cheeses. The PLS revealed that cheeses made at 90 DIM had a low content of FAA, while the difference between cheese ripened for 2 or 4 mo were less for the UNSAT and the CONTROL cheeses than for the SAT cheeses. Moreover, cheeses made at the mountain pasture had the best texture, most probably due to a higher TS content (Table 2).

5. Conclusion

In this study the effects of lactation stage and lipid supplemented concentrate on the composition of goat milk and its cheese making properties were investigated. Faster ripening and a better texture were obtained in cheese made of milk from goats that received concentrate supplemented with saturated lipids than cheese made from milk from goats that received the control concentrate or the concentrate supplemented with unsaturated fat. Lipid supplemented concentrate did not influence the casein content and or other main components in the goat milk. Stage of lactation influenced casein composition, content of lactose, protein, casein, NPN and calcium, and pH, SCC and the mean size of casein micelles.

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Figure legends

Figure 1. The content of individual caseins (g L^{-1} milk) throughout the lactation in milk from goats fed different lipid supplemented concentrates: CONTROL:—■—, saturated SAT: ••••♦••••, unsaturated UNSAT: - - -●- - -. The vertical line indicates the pre-experimental period (0-60 DIM) where all goats received the Control concentrate. Values for 30 and 60 DIM are means, and values for 120-230 DIM are least square means. DIM=Days in Milk.

Figure 2. Rennet clotting time (RCT) and curd firmness (a_{30}) in minutes and millimetres, respectively, throughout the lactation in milk from goats fed different lipid supplemented concentrates: CONTROL:—■—, saturated SAT: ••••♦••••, unsaturated UNSAT: - - -●- - -. The vertical line indicates the pre-experimental period (0-60 DIM) where all goats received the Control concentrate. Values for 30 and 60 DIM are means, and values for 90-230 DIM are least square means. DIM=Days in Milk.

Figure 3. Development a) of free amino acids (FAA) and b) Tyr and c) Trp after 2 and 4 moths of ripening of cheese made from milk from goats fed control concentrate (CONTROL) (black), concentrate with saturated fat (SAT) ▪(light grey) and concentrate with unsaturated fat (UNSAT) — (dark grey). Cheese made at 90, 120 and 190 DIM. Cheese made at 190 DIM was only analysed after 2 months of ripening due to small amounts of cheese. Tyr=Tyrosine, Trp=Tryptophane, DIM=Days in Milk

Figure 4. Loadings a) and scores b) of partial least square analysis (PLS2) of free amino acids (FAA) as X variables and sensory scores as Y variables during ripening of cheese made of milk from goats given diet with different fat sources. The first principal component (PC1) (X axis) explains 81 % and 6 % of the variation for the x and y variables respectively. PC2 (Y axis) explains 10 and 38 % of the variation of the x and y variables respectively. The cheeses are labelled according to DIM (90, 120

and 190), fat source in concentrate (C;control, U;unsaturated and S;saturated) and age of ripening (2 and 4 months). The attributes in between the ellipses of the loadings plot (a) contributes significantly to the variation. The ellipse in the score plot (b) surrounds the cheeses matured for 4 months.

Figure 1.

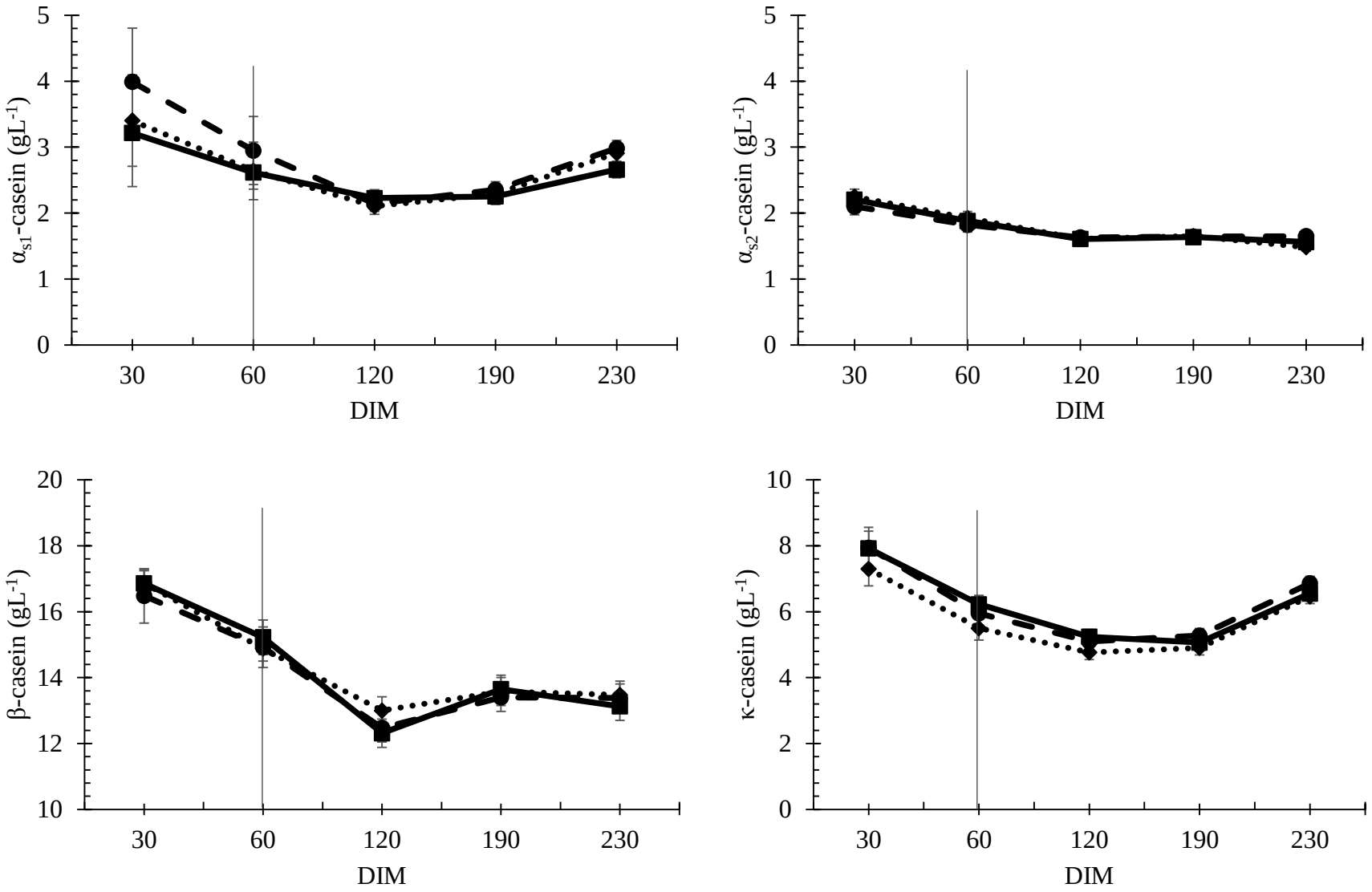

α_{s1}-casein (gL^{-1})
α_{s2}-casein (gL^{-1})
β-casein (gL^{-1})
κ-casein (gL^{-1})
DIM
30
60
120
190
230

Figure 2.

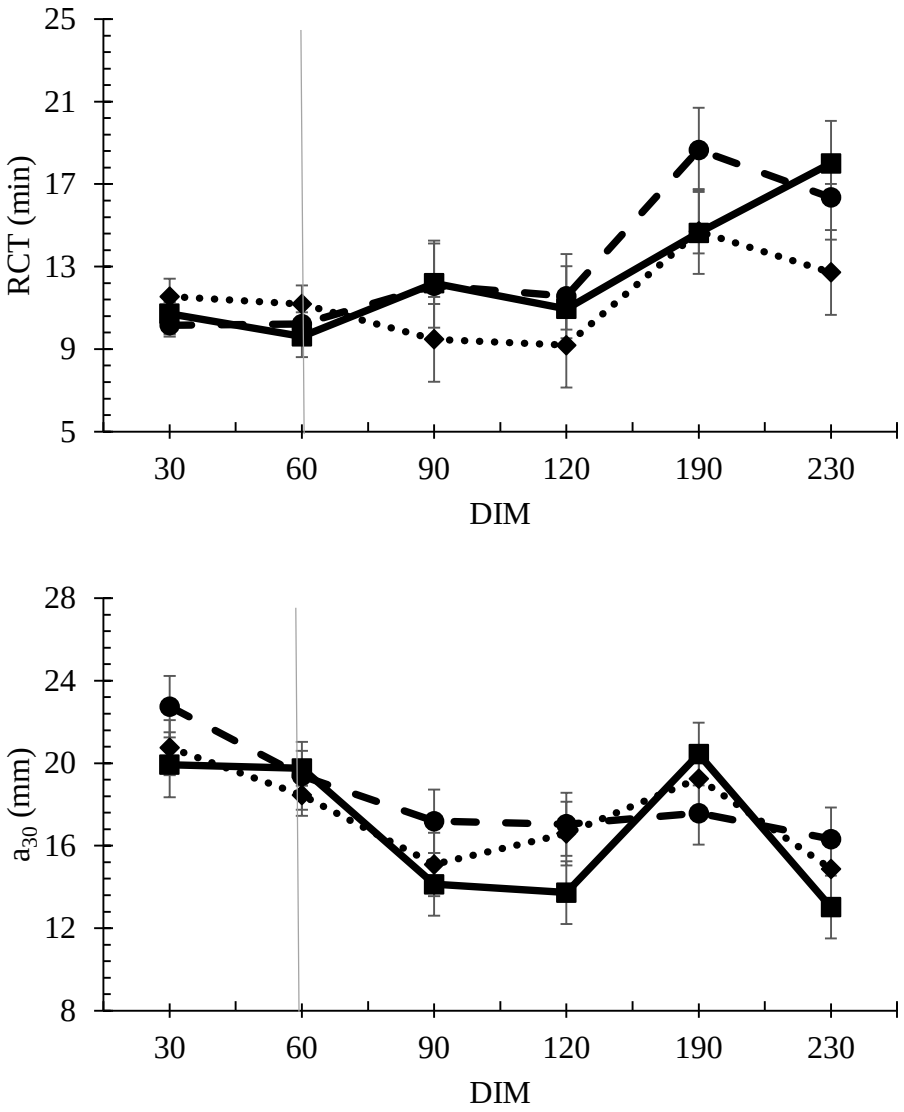
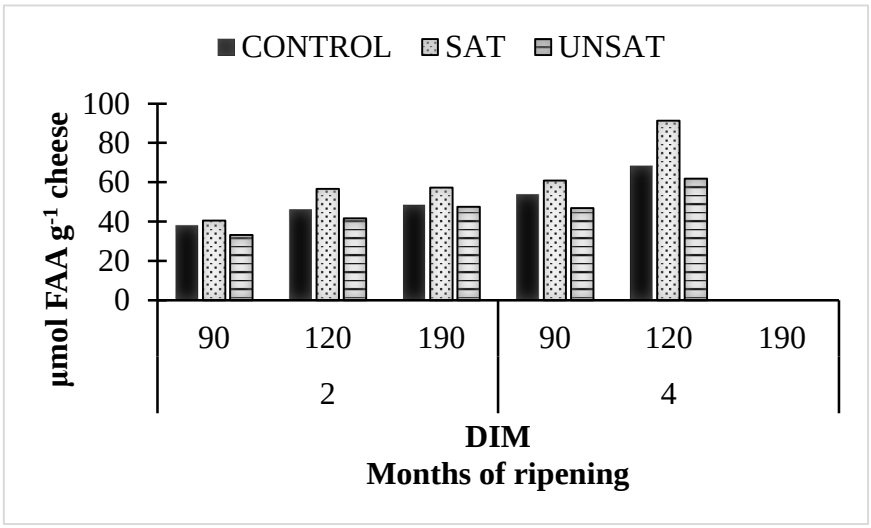
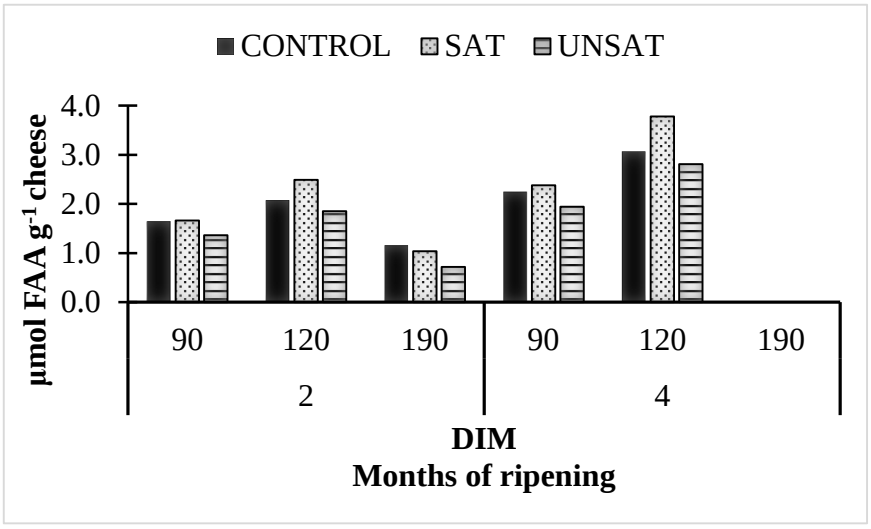

25
21
17
13
9
5
RCT (min)
30
60
90
120
190
230
DIM
28
24
20
16
12
8
a30 (mm)
30
60
90
120
190
230
DIM

Figure 3.

a)


CONTROL
SAT
UNSAT
µmol FAA g-1 cheese
100
80
60
40
20
0
90
120
190
90
120
190
2
4
DIM
Months of ripening

b)


CONTROL
SAT
UNSAT
µmol FAA g-1 cheese
4.0
3.0
2.0
1.0
0.0
90
120
190
90
120
190
2
4
DIM
Months of ripening

c)

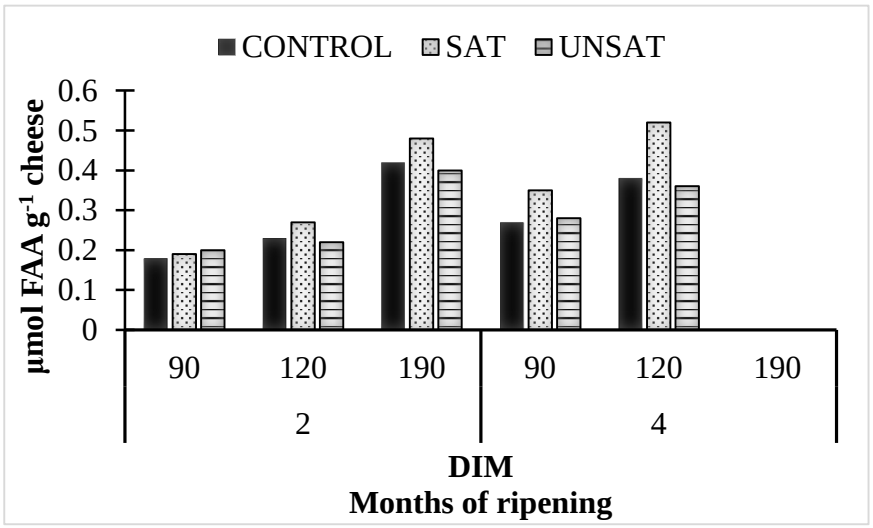
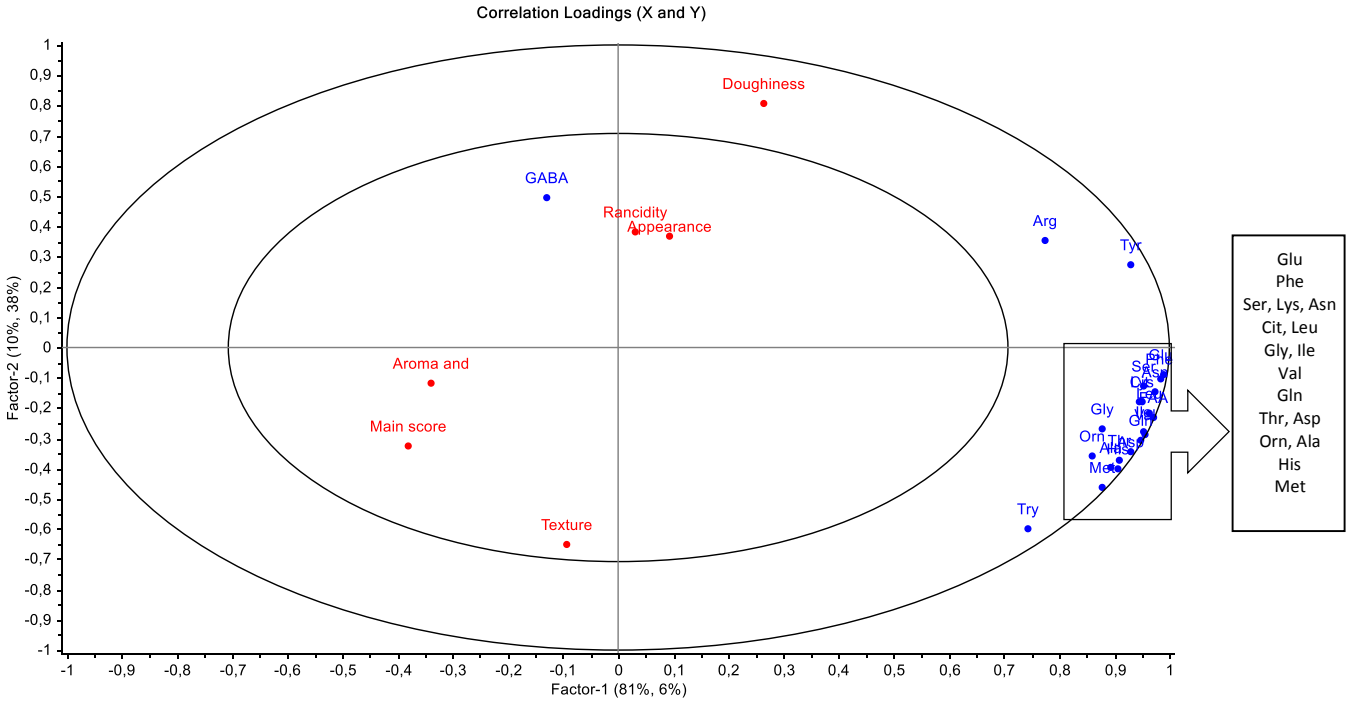

CONTROL
SAT
UNSAT
µmol FAA g-1 cheese
0.6
0.5
0.4
0.3
0.2
0.1
0
90
120
190
90
120
190
2
4
DIM
Months of ripening

Figure 4

a)


Correlation Loadings (X and Y)
Factor-2 (10%, 38%)
Factor-1 (81%, 6%)
Doughiness
GABA
Rancidity
Appearance
Arg
Tyr
Aroma and
Main score
Texture
Try
Gly
Glu
Phe
Ser, Lys, Asn
Cit, Leu
Gly, Ile
Val
Gln
Thr, Asp
Orn, Ala
His
Met

b)

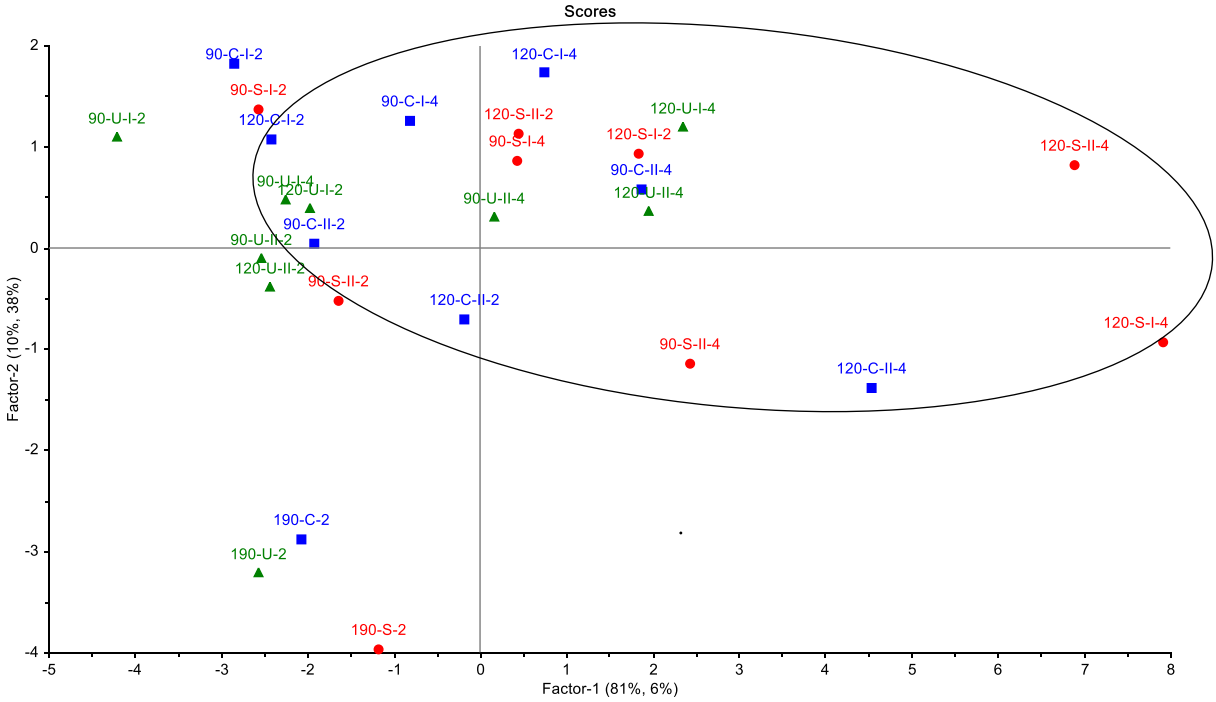

Scores
Factor-2 (10%, 38%)
Factor-1 (81%, 6%)
90-C-I-2
90-S-I-2
90-U-I-2
120-C-I-2
90-C-I-4
120-C-I-4
120-S-II-2
90-S-I-4
120-U-I-4
120-S-I-2
120-S-II-4
90-U-I-4
120-U-I-2
90-C-II-4
120-U-II-4
90-C-II-2
90-U-II-2
120-U-II-2
90-S-II-2
120-C-II-2
120-S-I-4
90-S-II-4
120-C-II-4
190-C-2
190-U-2
190-S-2

Table 1. Milk composition of goats fed different lipid supplemented concentrate (control (CONTROL), saturated (SAT) and unsaturated (UNSAT)) throughout the lactation. Values are given as means for 30-60 DIM (Days in milk) and least square means for 90-230 DIM. Different superscript (horizontally) letters indicates significantly ($P < 0.05$) differences within a row (DIM), and different subscript letters indicates significantly ($P < 0.05$) differences within a column (concentrate feed).

		Pre-experimental period		Experimental period				Level of Significance			
	DIM	30[1]	60[1]	90	120	190[2]	230	SEM	Feed	DIM	Feed* DIM
Analysis	Feed										
Lac	Control	4.91	4.63	4.31_b^x	4.30_b^x	4.12^y	4.03^y				
	Sat	4.82	4.56	4.29_b^x	4.33_b^x	4.01^y	4.00^y	0.06	ns	***	ns
	Unsat	4.84	4.61	4.48_a^x	4.50_a^x	4.07^y	4.08^y				
	SEM[3]	0.04	0.04								
LSCC	Control	5.10	5.34	5.23^z	5.34^z	6.98^x	6.13^y				
	Sat	4.87	4.96	5.30^z	5.35^z	6.93^x	6.10^y	0.30	ns	***	ns
	Unsat	5.28	5.51	6.04^{yz}	5.91^{yz}	7.56^x	6.22^y				
	SEM[3]	0.23	0.23								
pH	Control	6.77	6.60		6.53_a^z	6.77^x	6.70^y				
	Sat	6.76	6.62		6.63_b^z	6.82^x	6.72^y	0.03	ns	***	ns
	Unsat	6.71	6.55		6.66_b^z	6.85^x	6.73^y				
	SEM[3]	0.02	0.02								
TP	Control	3.22	2.89		2.40^z	2.93_{ab}^x	2.72^y				
	Sat	3.10	2.79		2.42^z	2.86_b^x	2.72^y	0.06	ns	***	ns
	Unsat	3.28	3.00		2.50^z	3.07_a^x	2.80^y				
	SEM[3]	0.09	0.06								
CN	Control	2.54	2.27		1.79^z	2.32^x	2.14^y				
	Sat	2.38	2.17		1.83^z	2.30^x	2.17^y	0.06	ns	***	ns
	Unsat	2.63	2.42		1.83^z	2.42^x	2.19^y				
	SEM[3]	0.08	0.06								
WP	Control	0.68	0.63		0.58	0.60	0.56				
	Sat	0.72	0.60		0.60	0.55	0.56	0.04	ns	ns	ns
	Unsat	0.64	0.59		0.66	0.65	0.61				
	SEM[3]	0.03	0.01								
NPN	Control	0.049	0.044		0.045^y	0.041^z	0.049^x				
	Sat	0.049	0.043		0.044^y	0.040^z	0.050^x	0.001	ns	***	ns
	Unsat	0.050	0.043		0.044^y	0.040^z	0.048^x				
	SEM[3]	0.002	0.001								
CN:WP	Control	3.83	3.63		3.27^y	3.91^x	3.88^x				
	Sat	3.53	3.72		3.34^y	4.21^x	4.04^x	0.21	ns	***	ns
	Unsat	4.24	4.16		2.87^y	3.85^x	3.62^x				
	SEM[3]	1.19	0.13								
Ca	Control	1.53	1.60		1.29_b^y	1.36^x	1.29^y				
	Sat	1.51	1.59		1.33_{ab}^x	1.31^{xy}	1.26^y	0.04	ns	***	**
	Unsat	1.56	1.63		1.43_a^x	1.40^x	1.24^y				
	SEM[3]	0.02	0.14								
CMS	Control	224	224		231^x	228^x	210^y				
	Sat	224	223		226^x	231^x	207^y	4.89	ns	***	ns
	Unsat	216	215		232^x	228^x	214^y				
	SEM[3]	3.73	3.43								

Ns:P > 0.05, *:P<0.05, **:P<0.01, ***:P<0.001

[1]All goats received the control concentrate.

[2]Mountain pasture.

[3]Standard error of mean for the pre-experimental period.

[a-c]Lsmeans within a column with different subscript letters differs (P<0.05).

[x-z]Lsmeans within a row with different superscript letters differs (P<0.05).

SEM: standard error of means, Lac: lactose, %, LSCC: log transformed somatic cell count, TP: Total protein, CN: casein, %, WP: Whey protein, %, NPN: non protein nitrogen, %, Ca: Calcium, g kg^{-1}, CMS: Casein micelle size, nm.

Table 2. . Least square means (LSM) and standard error (SE) of Total Solids (%), pH, the content of starter Lactococci ssp. (log cfu g^{-1} cheese), doughiness and texture during ripening of cheese made at tree stages during lactation from goats fed different lipid supplemented concentrates (unsaturated (UNSAT), saturated (SAT) and control (CONTROL)) . Significant differences ($P< 0.05$) within each factor is marked with different superscript letters.

Effect		Total Solids %		pH		*Lc.* spp.		Texture		Doughiness	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Group	CONTROL	49.52^{ab}	0.24	5.46	0.03	7.70^{a}	0.11	3.26^{a}	0.11	2.28^{b}	0.12
	SAT	50.16^{a}	0.24	5.51	0.03	7.33^{b}	0.11	3.19^{ab}	0.11	2.37^{b}	0.12
	UNSAT	49.04^{b}	0.24	5.45	0.03	7.35^{b}	0.11	2.90^{b}	0.11	2.78^{a}	0.12
DIM	90	48.90^{b}	0.20	5.36^{b}	0.02	7.34	0.08	2.90^{a}	0.09	3.01^{a}	0.09
	120	48.92^{b}	0.20	5.36^{b}	0.02	7.51	0.08	2.73^{a}	0.09	3.29^{a}	0.09
	190[1]	50.91^{a}	0.36	5.70^{a}	0.04	7.53	0.22	3.71^{b}	0.19	1.13^{b}	0.21
Mo	0	49.52	0.23	5.34^{b}	0.03	8.33^{a}	0.13				
	2	49.84	0.23	5.51^{a}	0.02	7.22^{b}	0.09	3.24^{a}	0.083	2.25^{b}	0.09
	4	49.37	0.28	5.58^{a}	0.03	6.83^{c}	0.13	2.99^{b}	0.11	2.70^{a}	0.12

Lc. spp: *Lactococcus,* LSM: least square means, SE: standard error, DIM: days in milk, Mo: months of ripening. ^{a,b}Lsmeans within a row with different superscript letters differs ($P<0.05$).[1] The results do not include cheese ripened for 4 mo.

Paper V

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The influence of the deletion in exon 12 of the gene encoding α_{s1}-casein (CSN1S1) in the milk of the Norwegian dairy goat breed on milk coagulation properties and cheese quality☆

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ABSTRACT

The influence on the cheese making properties and ripening of cheese made from goats' milk homozygous or heterozygous for the deletion in exon 12 of the gene encoding α_{s1}-casein (CSN1S1) of the Norwegian dairy goat breed was investigated. Milk from goats heterozygous for the deletion contained a high content of α_{s1}-CN compared with the milk from goats homozygous for the deletion that contained very low amounts of α_{s1}-CN. Milk from heterozygous goats contained the highest fat and protein content and exhibited the best coagulation properties; therefore, it was more preferable for cheese making. Cheese manufactured from milk from the heterozygous goats obtained a better and more stable cheese quality than did cheese made from the homozygous goats. The latter cheese had a higher moisture content, a more often rancid flavour and a different composition of free amino acids.

1. Introduction

Until 2007, the Norwegian dairy goats population exhibited a high frequency of goats with low or no synthesis of α_{s1}-casein, caused by an extremely high frequency (0.73) of a defective allele with a single nucleotide deletion in exon 12 of the gene encoding α_{s1}-casein (CSN1S1) (Dagnachew et al., 2011; Devold et al., 2011; Hayes et al., 2006). This deletion seems to be specific to the Norwegian dairy goat breed, and the deletion correlates with increased milk yield, reduced protein, fat and lactose content, a high content of free fatty acids (FFA) and a tart and rancid flavour (Dagnachew et al., 2011). The frequency of the deletion has been found to vary between 58% and 86% (Vegarud et al., 1999; Ådnøy et al., 2003). Later in this paper, goats homozygous for the deletion in exon 12 of the gene encoding α_{s1}-casein (CSN1S1) are denoted E12-00 goats, and goats heterozygous for the deletion are denoted E12-01 goats.

Previously, the Norwegian goat milk was mainly used to produce brown whey goat milk cheese, or "Brun Geitost", which has a distinctive sweet flavour with a slightly pungent rancid goaty background note balanced by the sweetness of the caramelised lactose. The goaty background note of this cheese was previously preferred by consumers until the late 1970s. Current consumers find this flavour less attractive. The rancid goaty flavour is considered a negative trait when manufacturing acid or rennet coagulating cheeses. During the 1960s and 1970s, the goaty flavour in the brown whey goat milk cheese

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became less apparent, and research and breeding were directed towards increasing the goaty flavour of brown whey goat milk cheese production (Skjevdal, 1979). This direction of breeding generated a high proportion of E12-00 goats (Ådnøy et al., 2003) and frequently a rancid and tart taste of the milk (Eknæs and Skeie, 2006). It also caused problems with coagulation, protein yield and sensory quality when producing acid and rennet coagulated cheeses. The result of the breeding strategy in Norway until 2007 (Norwegian Association of Sheep and Goat Breeders, 2013) was milk that was poor for producing rennet coagulated cheeses but acceptable for producing brown whey cheese. However, with decreasing demand for brown whey cheese and increasing demand for rennet or acid coagulated goat milk cheese, the status of the Norwegian goat stock had to be altered. A new breeding strategy directed towards E12-01/E12-11 goats with genes coding for the production of α_{S1}-casein was started in 2007 for the Norwegian dairy goat breed (Norwegian Association of Sheep and Goat Breeders, 2013).

A positive correlation between milk with a high content of α_{S1}-CN and good cheese making properties has been shown previously, whereas goat milk with a low content of α_{S1}-CN showed poor coagulation properties (Ambrosoli et al., 1988; Clark and Sherbon, 2000a; Pirisi et al., 1994). It has previously been shown that milk from the Norwegian E12-00 goats (E12-00 milk) requires a somewhat longer rennet clotting (RCT) and firming time (higher K_{20}) (Inglingstad et al., submitted for publication; Vegarud et al., 1999; Ådnøy et al., 2003) than do milk from goats heterozygous or homozygous (non-defective) (E12-01/11) for the deletion. The E12-00 milk produced a less firm or insufficiently firm coagulum after 30 min (A_{30}) and obtained a poorer syneresis than did milk from E12-01/11 goats (Inglingstad et al., 2014; Vegarud et al., 1999; Ådnøy et al., 2003).

As studies have not been conducted on how differences in the content of α_{S1}-CN in Norwegian goat milk caused by the deletion in Exon 12 influence cheese quality, the objective of this study was to investigate how these differences between E12-00 milk and E12-01 milk would influence cheese making and ripening, with a particular emphasis on the development of a rancid flavour during ripening.

2. Materials and methods

2.1. Animals and diets

The Norwegian University of Life Sciences has, alongside the breeding regime for E12-01/11, retained a few E12-00-goats in their stock. Therefore, two groups of goats of the Norwegian dairy breed could be designed: one group with eight E12-00-goats and one group with twelve E12-01-goats. The goats were genotyped for α_{S1}-CN, as described by Hayes et al. (2006). The average age of the goats was 3.5 years for the E12-00 goats and two years for the E12-01 goats. The average kidding date was February 16 ± 6 days in 2011 and was similar for the two groups. As the young goats have a lower milk yield than the older ones, the E12-01-group had to include four more goats than the E12-00-group to ensure sufficient milk for cheese production. All goats were fed the same diet during the experimental period.

2.2. Cheese manufacture process

Cheese was manufactured in two stages during early lactation; at approximately 30 (23–28) days in milk (DIM) and 60 (53–58) DIM. Two replicate blocks of cheese were made at each stage of DIM, with one day between each cycle (replicate block) of cheese making. Milk was collected in the evening two days before cheese production, in the morning and evening the day before, and in the morning the day of production. Cheese later described to be produced at 30 and 60 DIM was therefore made from milk collected from 23 to 28 or 53 to 58 DIM, respectively, and was produced at 25 and 28 or 55 and 58 DIM, respectively.

The raw milk was pasteurised at 72 °C for 15 s and was cooled to 32 °C, inoculated with 0.7% lactic acid bacteria starter (CHN19, Chr. Hansen A/S, Hørsholm, Denmark) and incubated for 25 min. The milk was added 0.01% of an adjunct, *Lactobacillus casei* TINE36 (Skeie et al., 2013) (yielding log 6.9 cfu g^{-1} of lactobacilli in the cheese at day 0) and was further incubated for 5 min before adding 25 mL rennet 100 L^{-1} milk (CHYMAX, Chr. Hansen A/S). The coagulum was cut in 10 mm cubes at appropriate firmness (clear cut), as evaluated by an experienced cheese maker. After cutting, the cheese curd was left to rest for 5 min and thereafter was stirred for 40 min at a constant temperature of 32 °C. The curd was washed by removing 50% (v/v) of the whey and then added 50% (v/v) of pasteurised water (32 °C). The curd was heated to 39 °C for 10 min and was then scalded for 30 min at this temperature. The cheese curd was transferred to Camembert type moulds (⌀ 11.5 cm). The cheese moulds were kept at 36 °C and were turned immediately after filling, i.e., after 30 min and 1 h. After 2 h, the cheese was cooled in water (10 °C) for 30 min and was then salted in saturated brine for 1 h. The cheese was dried overnight at 18 °C (room temperature) and vacuum packed in plastic foil (Cryovac, Oslo, Norway). The cheese ripened at 16 °C for two weeks and thereafter at 4 °C for the remaining ripening period.

2.3. Analysis of milk

The milk samples were collected from the evening and morning milk produced by each individual goat at 30 and 60 DIM, and the final sample comprised a pooled aliquot of milk from each of these two milkings (40:60 for the evening and morning milk). The content of free fatty acids (FFA), fat, lactose, protein and somatic cells were analysed in the milk produced by each goat with a Fourier Transform Infrared Spectroscopy (FTIR) (MilkoScan Combifoss 6500; Foss, Hillerød, Denmark). The milk samples for FTIR analysis were preserved with Bronopol (2-bromo-2-nitropropane-1,3-diol; D&F Inc., USA) and were kept at 4 °C until analysis. The pH of the milk (20 °C) was measured using a pH meter (PHM 61, Radiometer, Copenhagen) coupled to a pH electrode (pHC2005, Radiometer Analytical SAS, Villeurbanne Cedex, France).

The rennet clotting properties of the milk from the individual goats were analysed with a Formagraph (Lattodinamografo, Foss-Italia, Padova, Italy) according to McMahon and Brown (1982), measuring rennet clotting time (RCT), firming time (K_{20}) and gel strength (A_{30}). RCT was the time (min) from rennet addition until milk clotting started, as measured by an increased viscosity of the milk by the Formagraph. K_{20} was the time (min) from the start of clotting (RCT) until a width of 20 mm between the curves of the Formagraph was achieved. A_{30} indicates the distance (mm) between the curves of the Formagraph measured 30 min after the rennet addition. The milk was pasteurised (63 °C/30 min) and then cooled to 30 °C before 10 mL was transferred to the Formagraph sample cuvette and incubated at 32 °C. The rennet (200 μl CHYMAX, Chr. Hansen A/S, Hørsholm, Denmark) was diluted in an acetate buffer (1:50) and was added to the milk 30 min after incubation. The Formagraph was run for 30 min at 32 °C. All samples were run in triplicates. When no coagulation (RCT) occurred, a value of 50 min was assigned to the corresponding samples, and samples not achieving K_{20} were given a value of 40 min, following Devold et al. (2011).

The identification and quantification of individual caseins were analysed in milk from each individual goat with a Capillary Zone Electrophoresis (Agilent Technologies, Germany), following Mestawet et al. (2013). The quantification was performed using calibration curves of bovine casein standards (Sigma Aldrich). The total content of amino acids in the experimental milk was calculated using the amino acid composition for caprine casein retrieved from the UniProt database (www.uniprot.org).

2.4. Sampling, measurements and analysis of cheese

Samples were collected from pasteurised milk, from cheese 24 h after cheese making and from cheese ripened for two and four months.

Sampling for chemical and microbial analyses of cheese were conducted according to IDF-Standard 50C (IDF/FIL, 1995). Microbial counts, pH, organic acids and dry matter were analysed immediately after sampling, whereas free amino acids (FAA) were analysed later from grated cheese stored at −20 °C.

Presumptive *Lactobacillus* ssp. were enumerated on *Lactobacillus* selective agar (BBL™ LBS agar, Becton Dickinson and co., Le Pont de Claix, France) after anaerobic incubation in an anaerobic incubator (W.C. Heraeus GmbH, Hanau, Germany) with 10% (v/v) CO_2 for four days at 30 °C. Presumptive *Lactococcus* ssp. were enumerated on M17 broth (MERCK, Darmstadt, Germany) with 15 g L^{-1} Bactoagar (Saveen Werner AB, Malmø, Sweden) after aerobic incubation for two days at 30 °C. Coliform bacteria were enumerated on VRBA agar (OXOID, Hampshire, England) after incubation at 37 °C for 24 h.

Dry matter was determined according to IDF standard 4A (IDF/FIL, 1982). The pH of the samples was measured as described by Skeie et al. (2001) with an Orion pH-metre model 320 with an Orion Ross 8155 electrode (Orion Research, Cambridge, USA).

Free amino acids were analysed using HPLC with *O*-phthaldialdehyde (OPA) and fluorenylmethyl chloroformate (FMOC) derivatisation according to a modified method (Martinovic et al., 2013) of the previously described method by Bütikofer and Ardö (1999).

Organic acids were analysed using HPLC as described by Skeie et al. (1997) and with modifications as described by Skeie et al. (2008b).

A hedonic sensory evaluation of the experimental cheeses was performed by a trained panel of five assessors at the Norwegian University of Life Sciences after two and four months of ripening using a scale from 1 to 5, where 1 was not liked at all, and 5 was liked very much. The assessors evaluated their liking of the appearance, texture, flavour and taste and their overall likings of the cheese. If the cheese texture gained a score of 4 or lower, the extent of doughiness was graded from 1 to 5. If the cheese was given a flavour and taste score of 4 or lower, the intensity of tart and rancid flavour was graded from 1 to 5. The likings from the five assessors were averaged and used in subsequent calculations.

2.5. Statistical analysis

Significant differences ($P < 0.05$) between the fixed treatment factors of the milk (DIM and genotype) on milk composition and coagulation properties were determined by an analysis of variance using the ProcMixed procedure in SAS Enterprise Guide 5.1 (SAS Institute Inc., Cary, NC, USA) according to the following model:

$$Y_{ijk} = \mu + A_i + B_j + c(A_i) + \varepsilon_{ijk}$$

where μ is the intercept; A_i the fixed effect of genotype, with $i = 1,2$ (E12-00, E12-01); B_j is the fixed effect of lactation stage, $j = 1,2$ (30 DIM, 60 DIM); $c(A_i)$ is the random effect of goat within genotype, and ε_{ijk} is the experimental error.

Significant differences ($P < 0.05$) between the fixed treatment factors during cheese making and the ripening age on the dependent variables of the cheese were found by an analysis of variance for repeated measurements using the ProcMixed procedure in the SAS Enterprise Guide 5.1 according to the following model:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + A \times B_{(ij)} + d + \varepsilon_{ijkl}$$

where μ is the intercept; A_i the fixed effect of genotype, $i = 1,2$ (E12-00, E12-01); B_j is the fixed effect of lactation stage, $j = 1,2$ (30 DIM, 60 DIM); C_k is the fixed effect of ripening age, with $k = 1,2$ (two months, four months); $A \times B_{(ij)}$ is the interaction of genotype i and lactation stage j; d is the random effect of the two blocks; and ε_{ijkl} is the experimental error.

The covariance structure of the repeated measurements was chosen by comparing potential structures, using Akaike's and Schwarz's Bayesian information criterion (Wolfinger, 1996). A variance component structure proved useful for all data.

A principal component analysis of the FAA was conducted with The Unscrambler® X (CAMO Process AS, Oslo, Norway). The data were weighted by dividing each response variable by its standard deviation. A full cross-validation was used to validate the data set.

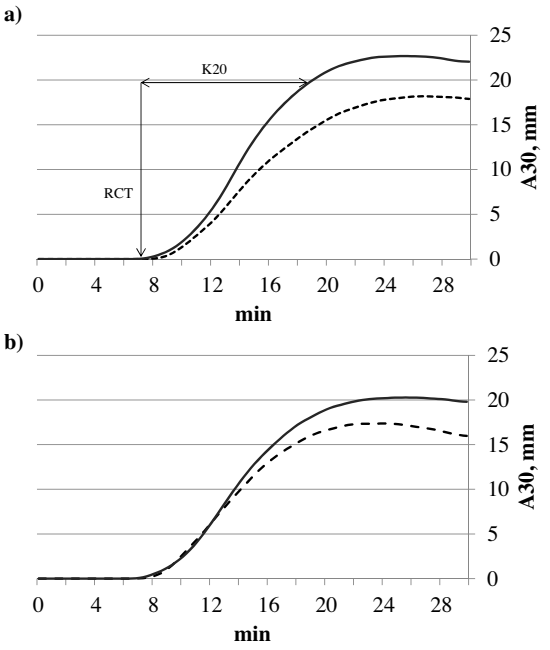

Fig. 1. The coagulation of the E12-00 (------) and E12-01 (——) milk on the Formagraph showing RCT (min), K_{20} (min) and A_{30} (mm) at (a) 30 DIM and (b) 60 DIM.

3. Results

3.1. Milk composition and coagulation properties

Compared with the E12-00 milk, the E12-01 milk had a higher protein content ($P < 0.001$) (Table 1). The pH and the content of fat, protein and lactose were lower ($P < 0.001$) in milk from 60 DIM than in milk from 30 DIM. No difference was detected in the number of somatic cells between the treatments, and this number averaged log 5.5 ± 5.7 cells mL^{-1} milk.

The E12-00 milk had a lower content of α_{S1}-CN ($P < 0.001$) and a higher content β-CN ($P < 0.05$) than the E12-01 milk, as shown in Table 1, whereas the content of α_{S1}-CN, κ-CN, β-CN and the sum of the individual caseins were higher ($P < 0.01$) in milk collected at 30 DIM than at 60 DIM. Although the casein composition differed between the experimental factors, the calculated total amino acid composition of the experimental milk seemed to be fairly similar, with less than a 0.1 g L^{-1} detected difference between the genotypes. The largest differences were found between 30 and 60 DIM for the content of Glu, Gln, Leu, Pro, Ser and Val (between 0.3 and 0.5 g L^{-1}) (results not shown).

The onset of coagulation (RCT) was not significantly influenced by the genotype (Fig. 1). A coagulum was obtained from all milk samples, but many samples did not achieve sufficient firmness with a width of 20 mm between the curves of the Formagraph (K_{20}). Specifically, 87% and 100% of the E12-00 milk at 30 and 60 DIM, respectively, and 33% and 41% of the E12-01 milk at 30 and 60 DIM, respectively, did not reach sufficient firmness (K_{20}) in some or all of the triplicate samples. The E12-01 milk obtained a

Table 1
The composition of goat milk from 30 and 60 days in milk (DIM) from goats with different deletions in exon 12 of the gene encoding α_{s1}-casein, specifically, goats homozygous for the deletion (E12-00) and goats heterozygous for the deletion (E12-01). Results are expressed as the mean values ± standard deviation. The statistical significance of DIM and genotype are indicated in the two columns to the right.

DIM		30		60		Statistical significance	
Genotype		E12-00	E12-01	E12-00	E12-01	DIM	Genotype
n		8	12	8	12		
Gross composition (%)	Fat	5.01 ± 0.68	5.33 ± 0.45	4.34 ± 0.45	4.63 ± 0.51	***	NS
	Protein	3.09 ± 0.23	3.45 ± 0.21	2.81 ± 0.22	3.06 ± 0.18	***	***
	Lactose	4.83 ± 0.18	4.93 ± 0.18	4.56 ± 0.15	4.62 ± 0.17	***	NS
Casein composition ($g\,L^{-1}$)	α_{S1}-CN	1.37 ± 0.51	3.95 ± 1.61	1.23 ± 0.46	3.03 ± 1.04	**	***
	α_{S2}-CN	2.37 ± 0.32	2.18 ± 0.42	2.07 ± 0.15	1.87 ± 0.36	***	NS
	β-CN	17.61 ± 0.62	16.56 ± 1.70	16.14 ± 1.08	14.54 ± 1.91	***	*
	κ-CN	7.69 ± 1.55	7.62 ± 1.24	6.63 ± 0.93	5.76 ± 0.95	***	NS
	SUM caseins	29.04 ± 1.94	30.32 ± 2.45	26.07 ± 1.67	25.22 ± 2.07	***	NS
FFA ($mmol\,L^{-1}$)		0.17 ± 0.14	0.10 ± 0.00	0.21 ± 0.15	0.14 ± 0.05	***	NS
pH		6.77 ± 0.12	6.76 ± 0.10	6.64 ± 0.10	6.58 ± 0.10	***	NS

NS: not significant.
n: number of goats.
FFA: free fatty acids.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

shorter firming time (K_{20}) and a higher gel-firmness (A_{30}) than did the E12-00 milk ($P<0.001$). The milk from 30 DIM produced a more ($P<0.001$) firm coagulum 30 min after rennet addition (A_{30}) than milk from 60 DIM.

3.2. Composition of cheese

As the cheeses were ripened in foil, the dry matter content did not change significantly during ripening. Cheese made from E12-01 milk (E12-01 cheese) had a higher ($P<0.001$) dry matter content (50.9 ± 0.6%) than did cheese made from E12-00 milk (E12-00 cheese) (48.1 ± 0.6%). Cheeses made at 60 DIM had a higher ($P<0.01$) dry matter content (49.8 ± 1.5%) than did cheeses made at 30 DIM (49.2 ± 1.5%). The pH and the microbial composition of the cheeses were not significantly influenced by the genotype (results not shown). Cheese made at 30 DIM had a 0.1–0.2 higher ($P<0.01$) pH and a lower ($P<0.05$) content of presumptive lactobacillus than did cheese made at 60 DIM (results not shown). Differences observed in presumptive lactobacilli were largest at the start of ripening (D0.4 cfu g^{-1} cheese at day 0, and D0.1 cfu g^{-1} cheese at four months).

3.3. Sensory quality

The assessors preferred the texture, the flavour and taste of the E12-01 cheese ($P<0.001$) to those of the E12-00 cheese, whereas no significant effect was found by DIM on the liking of these attributes (Table 2). The E12-00 cheese was found to be more ($P<0.001$) doughy and more rancid compared with the E12-01 cheese. Overall, the assessors preferred the E12-01 cheese ($P<0.05$) to the E12-00 cheese. The cheeses were more doughy ($P<0.01$) when produced at 60 DIM than at 30 DIM; moreover, an interaction ($P<0.05$) was shown by the E12-00 cheese being much more doughy than the E12-01 cheese at 60 DIM than at 30 DIM. The rancidity of the cheese increased ($P<0.001$) during ripening, and the two-months-ripened cheeses were preferred to the four-months-ripened ones.

3.4. Free amino acid (FAA) composition

The principal component analysis (PCA) of the FAA in cheese ripened for two and four months separated the treatments (Fig. 2), with the first principal component (PC1) discriminating the cheese with respect to age (86% of the variation explained) and the PC2 with respect to genotypes (7% of the variation explained). The PCA showed that the four-month-old cheeses had the highest content of FAA, and this result was significant ($P<0.05$) for all FAA without γ-amino butyric acid (GABA). The genotypes were clearly separated in PC 2, but only the content of Asn ($P<0.001$), Orn ($P<0.01$), Phe ($P<0.05$), Tyr ($P<0.05$) and Trp ($P<0.05$) were significantly influenced by the genotypes (Table 3), with Trp and Tyr being higher in the E12-00 cheese. This cheese was clustered above PC1, and the E12-01 cheese had a higher content of Asn, Orn and Phe and was clustered under PC1. DIM influenced ($P<0.05$) all FAA without Arg and Cit. Fig. 2 also shows the interaction effect ($P<0.05$) between the DIM and the genotype found for most of the FAA (without Asn, Cit, His and GABA), and a clear separation between DIM is shown for the E12-00 cheese, with cheese made at 30 DIM having a higher content of FAA, while the E12-01 cheeses were randomly distributed between the two stages of DIM along PC1.

3.5. Organic acids

Most organic acids developed similarly in the cheeses, thus indicating little influence from the experimental factors on the microbial metabolism during cheese ripening. However, the content of formic acid, which increased during ripening, was lower ($P<0.001$) in the E12-00 cheeses

Table 2
The sensory liking scores for cheese matured for two and four months. The cheese was made from the milk of goats at 30 and 60 days in milk (DIM). The goats had different deletions in exon 12 of the gene encoding α_{s1}-casein, specifically, goats homozygous for the deletion (E12-00) and goats heterozygous for the deletion (E12-01). The cheese was evaluated on a scale from 1 point indicating poor quality to 5 points indicating excellent quality. Results are expressed as the mean values ± standard deviation. The statistical significance of DIM, the genotype, the ripening age and the interaction between DIM and genotype are indicated beneath the results.

DIM		Appearance	Texture	Doughy	Flavour and taste	Rancidity	Main score
2 months of ripening							
30	E12-00	2.7 ± 0.1	2.8 ± 0.3	3.1 ± 0.1	2.9 ± 0.4	2.2 ± 0.6	2.7 ± 0.3
	E12-01	3.2 ± 0	3.1 ± 0.2	2.7 ± 1.4	3.2 ± 0	1.4 ± 0.3	3.0 ± 0.2
60	E12-00	3.5 ± 0.3	3.0 ± 0	4.6 ± 0.2	2.6 ± 0.4	2.1 ± 0.2	2.7 ± 0.3
	E12-01	3.1 ± 0.2	3.8 ± 0.3	3.3 ± 0.3	3.6 ± 0.2	1.1 ± 0.2	3.7 ± 0.3
4 months of ripening							
30	E12-00	2.8 ± 0.2	2.2 ± 0.2	3.1 ± 0.2	2.3 ± 0.5	3.8 ± 0.2	2.3 ± 0.5
	E12-01	3.2 ± 0.2	3.3 ± 0.1	2.7 ± 0	3.0 ± 0.5	2.5 ± 0.2	2.8 ± 0.7
60	E12-00	2.4 ± 0.2	1.8 ± 0	4.0 ± 0.7	1.7 ± 0.4	2.9 ± 0.5	1.6 ± 0.2
	E12-01	3.4 ± 0.5	3.1 ± 0.2	2.6 ± 0.2	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0
Statistical significance							
DIM		NS	NS	**	NS	NS	NS
Genotype		NS	***	***	**	***	*
Age		NS	**	NS	**	***	**
DIM*genotype		NS	NS	*	NS	NS	NS

NS: not significant.
n: number of judges.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 3
Free amino acids (μmol g^{-1} cheese) significantly influenced by the genotypes E12-00 and E12-01 and the sum of free amino acids (sum FAA). The cheese was made from the milk of goats at 30 and 60 days in milk (DIM), and the goats had different deletions in exon 12 of the gene encoding α_{s1}-casein, specifically, goats homozygous for the deletion (E12-00) and goats heterozygous for the deletion (E12-01). Results are expressed as the mean values ± standard deviation. The statistical significance of DIM, the genotype, the ripening age and the interaction between DIM and genotype are indicated beneath the results.

DIM		Asn	Tyr	Phe	Trp	Orn	Sum FAA
2 months ripening							
30	E12-00	1.5 ± 0.1	2.6 ± 0.2	3.6 ± 0.2	0.4 ± 0.0	1.9 ± 0.2	58.2 ± 3.1
	E12-01	1.8 ± 0.1	1.7 ± 0.2	3.5 ± 0.3	0.3 ± 0.0	2.0 ± 0.0	47.3 ± 5.1
60	E12-00	0.9 ± 0.1	1.6 ± 0.1	2.3 ± 0.2	0.2 ± 0.0	1.1 ± 0.1	30.5 ± 0.9
	E12-01	1.6 ± 0.4	1.4 ± 0.3	3.0 ± 0.5	0.2 ± 0.0	1.8 ± 0.4	37.6 ± 9.4
4 months ripening							
30	E12-00	2.0 ± 0.3	3.5 ± 0.4	4.8 ± 0.7	0.6 ± 0.1	2.8 ± 0.3	81.6 ± 14.0
	E12-01	2.6 ± 0.2	2.5 ± 0.4	4.8 ± 0.5	0.4 ± 0.1	2.9 ± 0.2	70.2 ± 9.7
60	E12-00	1.3 ± 0.0	2.3 ± 0.3	3.2 ± 0.0	0.2 ± 0.0	1.5 ± 0.2	44.0 ± 0.6
	E12-01	2.7 ± 0.5	2.6 ± 0.5	4.8 ± 0.8	0.3 ± 0.1	2.9 ± 0.6	65.2 ± 17.3
Statistical significance							
DIM		*	**	**	***	**	***
Genotype		***	*	*	*	**	NS
Age		***	***	***	**	***	***
DIM*genotype		NS	**	*	**	**	*

NS: not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

than in the E12-01 cheeses (Fig. 3). The content of uric acid was the highest ($P < 0.001$) in the 24 h cheeses and was higher ($P < 0.05$) in the E12-01 cheese than in the E12-00 cheese. The content decreased during ripening, and uric acid was not detected in cheese after four months of ripening, thereby indicating that the content of uric acid was different in the milk initially.

4. Discussion

Until now, only studies of the initial cheese making properties (milk composition, coagulation properties and syneresis) of milk from the different genotypes of the Norwegian goats had been conducted (Devold et al., 2011; Vegarud et al., 1999). This is the first study to include

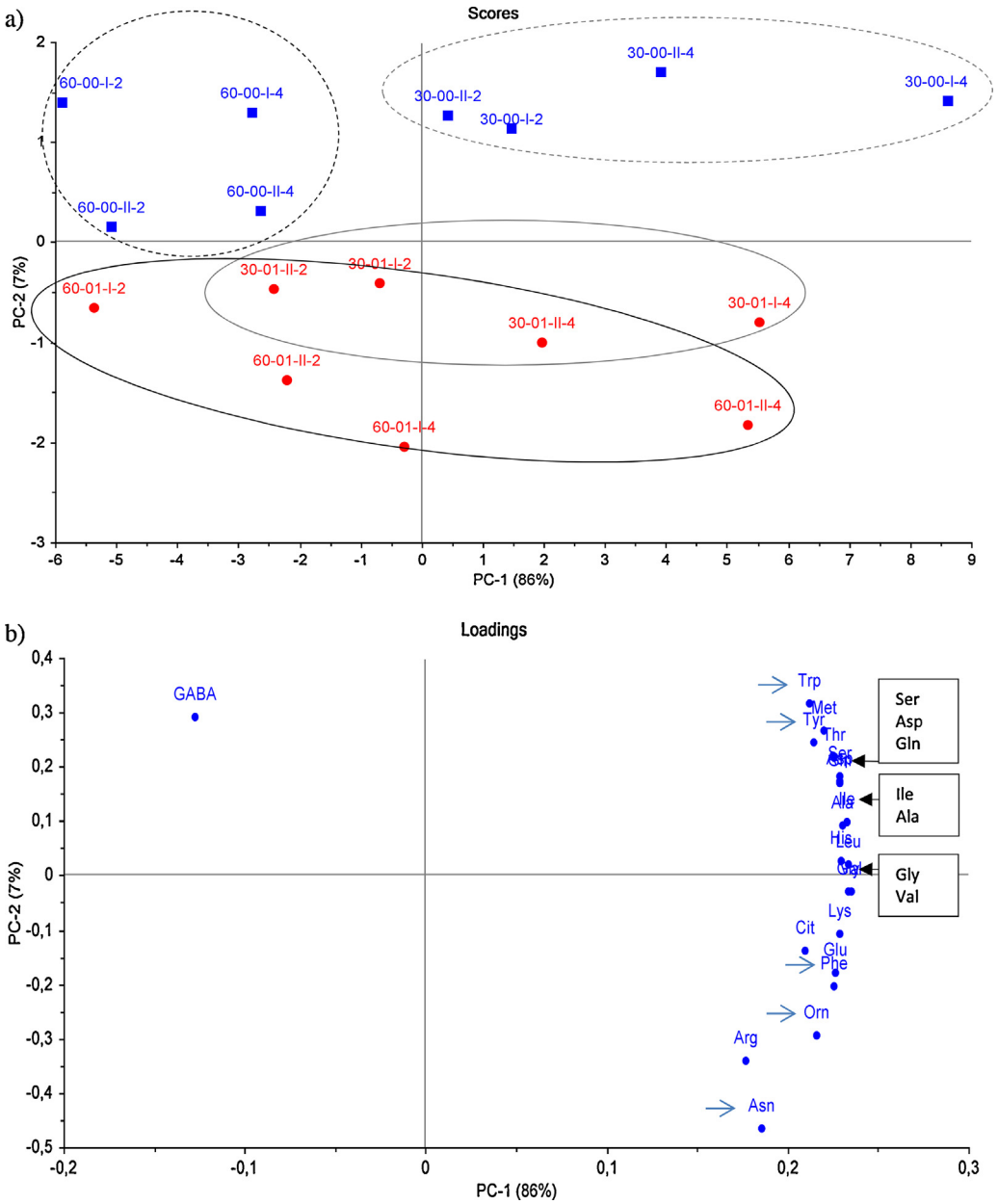

Fig. 2. The scores (a) and the loading (b) plot of the PCA of free amino acids during the ripening of cheese made from E12-00 milk and E12-01 milk, where 86% and 7% of the variations were explained by the first two components. The cheeses are labelled according to the days in milk (DIM) (30 DIM and 60 DIM), the genotype (00 and 01), the replicate block (I and II) and the age (two and four months). The dotted circles surround the E12-00-cheeses, and the solid circle surrounds the E12-01-cheese. The grey circles surround cheeses manufactured at 30 DIM, and the black circles surround cheeses manufactured at 60 DIM.

the complete cheese making and ripening of cheese made from the milk of these goats. Consistent with previous findings, the coagulation properties of milk with the highest content of α_{S1}-CN was superior compared to those of milk produced by goats having weak genotypes for α_{S1}-CN (Ambrosoli et al., 1988; Clark and Sherbon, 2000a; Pirisi et al., 1994). The casein composition of the milk from the Norwegian goat genotypes has been surveyed by capillary electrophoresis in two recent studies (Inglingstad, personal communication; Inglingstad et al., 2014), and only small quantities of α_{S1}-CN were found in the E12-00 milk. The content of α_{S1}-CN in the Norwegian E12-00 milk was, however, higher than that reported in milk from other goat breeds homozygous for a deletion in the gene encoding for α_{S1}-CN (Pierre et al., 1999; Tziboula and Horne, 1999).

The average RCT was similar for the two types of milk used in this experiment, whereas other studies have found differences between different genotypes. For milk from goats with weak genotypes for α_{S1}-CN, both longer (Devold et al., 2011; Inglingstad et al., 2014; Vegarud et al., 1999)

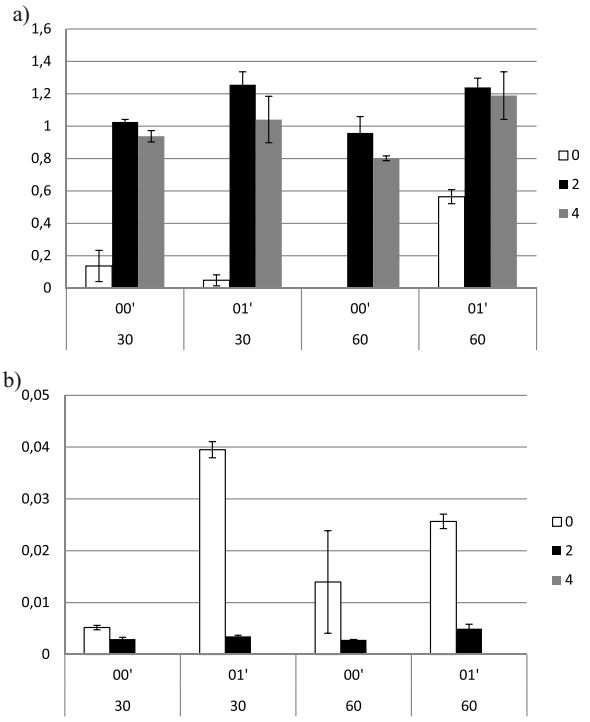

Fig. 3. The development of formic acid (a) and uric acid (b) (mmol g^{-1} cheese, mean ± SD) during the ripening of cheese made from E12-00 milk and E12-01 milk. The cheeses are labelled according to the days in milk (DIM), 30 and 60; the genotype, 00 and 01; and the age, zero (white bar), two (black bar) and four (grey bar) months of ripening.

and shorter (Ambrosoli et al., 1988; Clark and Sherbon, 2000b) RCTs have been reported, compared with milk from goats with stronger genotypes. The over frequency of a poor coagulum made by the E12-00 milk is consistent with the above-mentioned findings.

The E12-00 cheese had a lower dry matter content, thus implying that this cheese matrix trapped more water. This result might be explained by the presence of larger casein micelles in the milk with a low content of α_{S1}-CN, as this milk also had a higher content of β-CN. An increased casein micelle size has been found in Norwegian goat milk lacking or having a low content of α_{S1}-CN (Devold et al., 2011; Inglingstad et al., 2014; Ådnøy et al., 2003). The interaction between the serine-phosphate groups and calcium ions (Ca^{2+}) in α_{S1}-CN, α_{S2}-CN and β-CN is strong, and contribute, along with hydrophobic and other weak interactions between the caseins, to the formation of the casein micelle (Dalgleish, 2011). The β-CN is highly voluminous and hydrophobic, displays amphipathic properties and favours the formation of highly hydrated casein micelles (Dalgleish, 2011).

During cheese ripening, the caseins are degraded by the enzymatic action of rennet, lactic acid bacteria (LAB) and indigenous enzymes (i.e., plasmin). The content of FAA in the cheese can be used as a ripening index, as the FAA are products of the proteolytic activity in the cheese (Fox et al., 2000). The proteolysis is considered the most important biochemical event that occurs in cheese during ripening, as it is responsible for textural changes and flavour development during ripening. The genotype was crucial for the development of some of the FAA. By calculating the total content of these amino acids in the (present) individual caseins using the amino acid composition for goat casein given by UniProt (www.uniprot.org), no significant difference was detected between the two genotypes with respect to the milk's total amino acid content. Balia et al. (2013) showed that the amino acid composition varied to some extent among different genetic variants of α_{S1}-CN, and the Norwegian deletion in exon 12 (Lien, 1995) might produce a truncated protein lacking approximately one third of the amino acids, compared with the original α_{S1}-CN. The cause of the differences in the content of FAA between the cheeses made from milk with different α_{S1}-CN genotypes therefore requires further investigation.

The E12-00 cheese contained more β-CN and more moisture, whereas the E12-01 cheese contained more α_{S1}-CN and less moisture. These compositional differences in turn would likely influence the activity of the ripening enzymes, as most of these enzymes are highly specific. In semi hard cheeses, plasmin is the most important protease for the degradation of β-CN, whereas rennet is the most important for the degradation of α_{S1}-CN (Sousa et al., 2001). The content of free Trp and Tyr were significantly higher in the E12-00 cheese than in the E12-01 cheese. According to our calculations, the content of these amino acids in the caseins of the cheeses did not differ. However, Trp and Tyr are present at more of the cleavage sites of plasmin and rennet on α_{S1}-CN than on β-CN (Upadhyay et al., 2004), and a higher content of these FAA were therefore expected in the cheese with the highest content of α_{S1}-CN. In addition, other authors (Hayaloglu et al., 2013; Teiada et al., 2008) found little proteolysis of β-CN during ripening of goat milk cheeses, and α_S-CN was extensively degraded. The water activity is an important regulator of the biochemical activity in cheese, and the difference in moisture content between the E12-00 and E12-01 cheeses appears to have been the most important explanation for the differences in enzymatic activities and thus, the differences in individual FAA in the cheeses.

The E12-01 cheese ripened for four months had a higher content of FAA than did cheese ripened for two months, as expected. However, the E12-00 cheese did not develop in this way, such that the stage of lactation (DIM) was more important for the development of FAA than the ripening time. Cheese made from milk at 30 DIM had a higher content of FAA than did similar cheese made from milk at 60 DIM, independent of age of ripening. These results may indicate that the ripening enzymes had different conditions for their activity in the E12-00 cheeses at the two stages of early lactation, and the conditions likely were highly similar at the two stages of lactation for the E12-01 cheeses. The E12-00 cheeses made at 30 DIM had a 1% higher moisture content than did the E12-00 cheeses made at 60 DIM, and the proteolytic activity normally increase with increased moisture content. However, the E12-01 cheeses had a 2–3% lower moisture content than did the E12-00 cheeses but a higher content of FAA than did the E12-00 cheese made at 60 DIM. Differences in the flora of non-starter LAB (NSLAB) could also be a plausible explanation for differences in the content of FAA due to DIM. However, the cheeses were all

added an adjunct *Lb. casei* to oust the NSLAB, and the development of the organic acids did not indicate significant differences in the metabolic activity of the bacteria present in the cheeses. The formic acid was likely derived from the degradation of FAA by the lactobacilli in the cheese, as its content increased during cheese ripening (Skeie et al., 2001, 2008a).

Plasmin is an indigenous milk enzyme important for the degradation of β-CN into peptides in cheese, and β-CN might be further degraded to FAA by the enzymatic action of LAB. Plasmin and its precursor plasminogen are associated with the casein micelles in milk. Cortellino et al. (2006) and Fantuz et al. (2001) found that in goat milk, the plasmin activity was higher and the plasminogen activity was lower compared with bovine and ovine milk. Moreover, Cortellino et al. (2006) found the plasmin activity in goat milk to be higher in late lactation than in early lactation. The present experiment was conducted in early lactation, and the content of β-CN and the total casein in milk was lower at 60 DIM than at 30 DIM; therefore, the lower content of β-CN likely resulted from a lower total milk casein content, not differences in plasmin activity.

The cheese made in this study was a model cheese and generally obtained a low liking score by the sensorial panel. Nonetheless, a significant difference in the sensory liking between cheeses made of milk from the two genotypes was obtained. The most distinguished characteristic was the rancid taste of the cheese made from the E12-00 milk. The frequently rancid and tart flavour of Norwegian goat milk (Eknæs and Skeie, 2006) results from the lipolysis of milk fat into free fatty acids (Collins et al., 2003). Therefore, the rate of lipolysis was likely provoked in the E12-00 cheese, and more FFA could be released. Delacroix-Buchet et al. (1996) found a higher lipase activity and a higher content of total FFA in goat milk with a low content of α_{S1}-CN, compared with goat milk with a high content of α_{S1}-CN. In the present experiment, cheese was made from pasteurised milk, and the indigenous lipases were expected to be inactivated. However, differences in the composition and the stability of the fat globule membrane might explain this result and should be further investigated.

Delacroix-Buchet et al. (1996) found that cheese made from milk with a low content of α_{S1}-CN had a less firm texture and a more pronounced goaty flavour than did cheese made from goat milk with high α_{S1}-CN content. This finding is consistent with the results of the present experiment, where the sensory analysis showed a clear difference between the cheeses made from milk of the two genotypes. The quality of the E12-01 cheese was mostly stable independently of stage of lactation, while the quality of the E12-00 cheese did depend on the stage of lactation investigated, even though the two investigated stages were in the early phase of lactation. Soryal et al. (2005) found that the breed and the casein content of milk influenced how the flavour and texture score of soft cheese was influenced by lactation. The sensory score of cheese produced from milk of the Alpine breed, milk with a low casein content, was influenced by lactation, and cheese produced from milk from the Nubian breed, milk that has a high casein content, underwent no significant changes in quality during lactation.

5. Conclusion

This experiment confirmed that the cheese making properties and the cheese quality of milk from goats heterozygous for the deletion in exon 12 and with a high content of α_{S1}-CN was superior to milk from goats homozygous for the deletion in exon 12 and with very low amounts of α_{S1}-CN. Cheese made from milk with the highest α_{S1}-CN content achieved a better and more stable cheese quality. Cheese made from milk with a low content of α_{S1}-CN had a higher moisture content, which implies that the cheese matrix trapped more water. Furthermore, milk from homozygous goats yielded cheese more often with a rancid flavour, a different composition of FAA and its cheese making properties and cheese quality more dependent on DIM.

Conflicts of Interest

None.

Acknowledgements

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